Intraspecific variation in seed dormancy and germination among populations of *Stellaria media* and *Galium aparine*

- A modelling approach to investigate the effects of environmental and management factors

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Abstract

Weeds are a substantial threat to arable crop production, reducing crop yields and/or reducing crop quality and market value. Worldwide expenditure on herbicides outstrips that on either insecticides or fungicides. However falling grain prices are causing farmers to reconsider the economics of herbicide use and public concern about countryside conservation and demand for organically produced food is generating increased pressure to minimise pesticide use. With increasing interest in more integrated approaches to weed control there is a need to better understand the ecology and biology of weed species. This thesis therefore aims to describe aspects of the ecology of two common winter annual weed species, *Stellaria media* and *Galium aparine*, in order to support the development of more sustainable weed control measures in arable crops. As effective broad-leaved weed control tends to be associated with early removal of weed seedlings, this thesis adopts a modelling approach to investigate the potential benefits derived from predications of weed seedling emergence.

Seedling emergence can be divided into three stages: seed dormancy, seed germination and seedling pre-emergence growth. As seed dormancy and germination are particularly variable this thesis focuses on these key processes. Models for the timing and extent of germination for *S. media* and *G. aparine* are developed and variability is addressed by investigating the extent of intraspecific variation between populations and according to maternal effects. Intraspecific variation between populations was investigated using contrasting populations selected from a large-scale screening.

The seed dormancy and germination models were developed following a review of existing models and identification of data requirements. This initiated field experiments to quantify seasonal changes in *S. media* seed dormancy and laboratory experiments to quantify the combined effects of temperature and water potential on seed germination for *S. media* and *G. aparine*. Also according to the data requirements identified by review, further laboratory experiments were conducted to analyse the effects of light and nitrate on *S. media* germination. For *G. aparine*, additional data were derived, as required, from published literature.

For *S. media* significant variation between populations was recorded in initial and seasonal patterns of seed dormancy, and in the timing and extent of seed germination according to temperature, water potential and light. For *G. aparine* significant variation was recorded between populations for initial seed dormancy and seed germination according to temperature and water potential.

In general, for both *S. media* and *G. aparine*, seed dormancy was released in autumn and induced in early summer. Dormancy induction in the summer for *S. media* was complete but for *G. aparine*, restricted germination to a narrower range of temperatures. Optimum temperatures for germination of *S. media* tended to 20 °C and for *G. aparine* to 10 °C (range assessed 5 to 30 °C). Germination extent decreased and the time to germination increased as water potential decreased, but *G. aparine* was relatively more resilient to reduced water potential over the range 0 to -0.4 MPa, at optimal and sub-optimal temperatures (range assessed 0 to –0.8 MPa).

The timing and extent of germination in the soil seedbank was modelled in terms of the factors influencing dormancy (soil temperature) and germination (temperature and water potential). The dormancy models were developed from existing models for summer annual species. The germination models were developed independently following examination and assessment of existing hydrothermal time models. Both models were developed in a stochastic framework and parameters were estimated separately for the different populations.

Maternal effects were not included in the model, but experiments showed this additional variation would also significantly impact on model application. This was illustrated using seeds produced following application of the herbicide, fluroxypyr, at a range of reduced rates. For *S. media* the effect of fluroxypyr application on subsequent seed germination varied between populations, and higher rates reduced the extent of seed germination for two of the three populations. For *G. aparine* effects similarly varied between populations, though the higher rates of fluroxypyr application consistently reduced the extent of seed germination and lengthened the timing of germination. For *S. media* additional experiments investigating the effect of nitrogen environment during seed production showed no significant effects on subsequent seed germination.

Variability between populations and variability between seeds produced in different maternal environments complicates the task of predicting the extent and timing of *S. media* and *G. aparine* germination. However these models do serve a useful role as a research tool in summarising current understanding of how different environmental and management factors may effect weed seed dormancy and germination.

Declaration

This thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported within was executed by me, unless otherwise stated.

April, 2000

Dedication

To Glenn, for his love, encouragement and long-suffering patience.

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Chapter 1. Introduction

'A weed is a plant growing where it is not wanted.'
(Mortimer, 1990).

In arable crops, weeds are plants that reduce yield, increase costs and/or reduce the quality and market value of the crop. Reduction in crop yield occurs either directly through crop-weed competition for resources such as light, nutrients and water or indirectly as some weeds are hosts to plant pathogens, viruses and insect pests. Increased production costs occur when additional cultivations are required to limit weed establishment or when weeds interfere with harvesting, increasing the time and effort required to harvest the crop. Reduction in crop quality and market value occurs through crop contamination with weed seeds, or through reduction in crop qualities such as grain size or nitrogen content.

The importance of weed control is illustrated by the fact that worldwide expenditure on herbicides outstrips that on either insecticides or fungicides (Mortimer, 1990) and herbicide use is the main method of weed control employed in arable crop production in the United Kingdom (Tottman & Wilson, 1990). Falling grain prices have caused farmers to reconsider the economics of herbicide use and increasing instances of herbicide resistance (Attwood, 1985; Cousens & Mortimer, 1995) have caused farmers to re-evaluate current weed control programmes. At the same time, public concern about countryside conservation and demand for organically produced food has generated increased pressure to minimise pesticide use (Tottman & Wilson, 1990). This means that there is increasing interest in integrated weed management, incorporating more targeted use of low dose herbicides and methods of non-chemical weed control. It is clear that this approach requires better understanding of the ecology and biology of weed species in order to identify the most appropriate measures and timings for effective weed control.

It is the aim of this thesis to better understand the ecology of two common winter annual weed species, *Stellaria media* (L.) Vill. and *Galium aparine* L., particularly with regard to the early stages of the plant life cycle where understanding can best support the development and application of alternative weed control methods in arable crops.

1.1. Weeds and ecology

Ecology is the scientific study of the interactions that determine the distribution and abundance of organisms (Krebs, 1972). Applied to weeds, these interactions include biotic factors such as other weed and crop plants and abiotic factors such as weather and soil conditions. Consequently, the aims of weed ecology are to explain and predict the distribution and abundance of weeds in relation to such environmental factors.

These are ambitious aims and it has been argued that to date, ecology has singularly failed to deliver adequate predictions regarding the distribution or abundance of weeds in crop rotations (Cousens & Mortimer, 1995). This has been attributed both to limitations in the extent of relevant information available and the failure to use existing information to formulate appropriate models to describe the processes that determine when, where and the extent of weed problems that occur (Cousens & Mortimer, 1995).

At first sight, the literature concerning weed species is extensive. However closer examination reveals that much of the research on weed species has concerned the development and testing of herbicide compounds and that with few exceptions, notably *Avena fatua* and *Alopecurus myosuroides*, efforts to fully describe the ecology of weed species have been limited. Even where experiments have detailed aspects of weed ecology, such as seed persistence in the soil or seed germination responses to light, it is clear that results in the literature are often contradictory. It is thought that this may reflect variation between the populations studied or variation in the local environment or test conditions. It is clear that the environment in which the weed seeds are produced can have profound effects on the characteristics of both seeds and their resulting seedlings (Gutterman, 1992).

1.1.1. Variation between weed populations

The majority of studies concerned with weed ecology have focussed investigations on single populations and attempted to generalise patterns and processes from these observations. This ignores the observed fact (e.g. Chancellor & Peters, 1972; Froud-Williams & Ferris-Kaan, 1991) that pronounced differences can exist between populations in characteristics such as plant size, growth habits, phenology, seed dormancy and germination requirements.

Differences between weed populations have most commonly been described for populations that differ in susceptibility to a particular herbicide. For example, Hill, Courtney & Harvey

(1991) showed differences in plant susceptibility to the herbicide, fluroxypyr for populations of *Galium aparine*. Warwick & Marriage (1982a, b) showed population differences in phenology and growth characteristics between atrazine-resistant and atrazine-susceptible populations of *Chenopodium album*. Similarly Boutsalis & Powles (1998) showed differences between herbicide resistant and herbicide susceptible populations of *Sisymbrium orientale*; Kremer & Lotz (1998a, b) for populations of *Solanum nigrum* and Hall & Romano (1995) for populations of *Sinapis arvensis*.

Whilst the evolution of herbicide resistance has attracted most interest and concern, population differences have also been shown to exist for weed populations growing in different habitats. For example, Froud-Williams (1985), Ferris (1988), Bain & Attridge (1988) and van der Weide (1992, 1993) have all demonstrated differences between arable and hedgerow populations of *Galium aparine* for characteristics including plant morphology, flowering phenology and germination requirements. However it was also evident that there was substantial variation within the habitat groupings, for field and hedge populations collected from different geographical locations.

Whilst the evolution of differences between populations from different habitats and geographical locations is perhaps not surprising, population differentiation has also been demonstrated on a local scale. Most notably, van der Vegte (1978) showed differences between two populations of *Stellaria media* growing in the same field in the Netherlands. It was shown that the populations differed markedly in their phenology, seed dormancy and seed germination requirements such that one of the populations built up a relatively uniform short lived seedbank, whilst the other built up a persistent seedbank of phenotypically diverse seeds.

It is clear from this discussion, that substantial variation can exist between populations and that this variation can exist at a range of scales. The extent of this variation may in part explain some of the contradictory information published on weed ecology. For these reasons, care should be taken in interpreting and extrapolating from results based on the study of a single population. This would also seem to suggest that future experiments to investigate aspects of weed ecology should include a number of populations and that this would thereby support the generality of the patterns and processes described.

1.1.2. Variation according to differences in the maternal environment

Studies of population differences are complicated by the persistence of environmental effects during seed development. These differences have commonly been termed 'maternal effects', though Baskin & Baskin (1998) suggested that such effects were better described as 'pre-conditioning' effects in order to distinguish between heritable variation derived from the maternal genotype and variation related to environmental differences during seed formation. Whilst it is accepted that this may be a useful distinction in some circumstances, these effects will remain termed 'maternal effects' in this thesis. This is for reasons of consistency with other work and because it is recognised that in this thesis, observed variation between seeds produced in different conditions results from the interaction between maternal genes and the maternal environment and these two factors cannot be readily distinguished.

There are however circumstances in which we are concerned to limit the effect of the maternal environment. For example, if we are concerned with describing differences between populations, it is important that these differences are largely fixed and consistent, such that the differences described are robust and reliable. That is, we are interested in the genetic characteristics of the population. The results of such experiments, especially when conducted with weed seeds or seedlings, can be confounded by differences within and between seed populations related to environmental conditions during seed production (Gutterman, 1980, 1992; Wulff, 1995; Roach & Wulff, 1987). Consequently it is desirable to limit the differences in environmental conditions during seed formation when comparing populations. It is, however, notable that few attempts have been made to control for this factor.

Roach & Wulff (1987) suggested that this effect could largely be minimised by growing plants from different environments in a common environment for one generation, and using this next seed generation as the basis for study of the differences between populations. Quinn & Colosi (1977) also advocated this approach. This approach assumes that most of these effects are short lived and not persistent, though there is only limited evidence to support this (Roach & Wulff, 1987). Maternal effects are not only related to large scale differences in climate and edaphic factors, but can also be related to differences in the microclimate experienced by different seeds on the same parent plant (Kane & Cavers, 1992).

The previous discussion has illustrated that there are inherent genetic differences between populations and that these interact with differences related to environmental conditions during seed production. As such, it is a central concern of this thesis that all investigations should describe the extent of variation within and between populations. The observation that these maternal effects are most apparent as they affect seed dormancy, germination and seedling characteristics is particularly problematic for this study (Roach & Wulff, 1987). This is because in developing alternative strategies of weed control, the focus of our interest necessarily concerns the seed and seedling stages of the weed life cycle.

1.2. Prediction of weed seedling emergence

It has been argued that understanding of the patterns of weed seedling emergence is critical in weed control decision-making (Spitters; 1989; Alm, Stoller & Wax, 1993; Harvey & Forcella, 1993; Ghersa & Holt, 1995; Grundy, Mead & Burston, 1999). Many arable weeds are annual species that form persistent seed banks (Thompson & Grime, 1979; Grime, 1989; Mortimer, 1990) and following soil cultivation, to establish the arable crop, a variable proportion of these weeds will germinate and emerge. It has been shown that both the timing of weed seedling emergence relative to the crop and the number of weed seedlings emerging are important in determining the levels of crop-weed competition (Benjamin, 1990; Kropff *et al.*, 1992). It has also been shown that low dose herbicide applications and cultural methods of weed control tend to be more effective when applied early in the growing season, against smaller weed seedlings (Kudsk, 1989; Whytock & Davies, 1996). As such, the ability to predict weed seedling emergence from knowledge of the soil seedbank may be a useful tool in developing targeted and timely weed control measures in organic and integrated farming systems.

Previous efforts to predict weed seedling establishment from knowledge of the soil seedbank have largely proven unsuccessful (Forcella, 1992; Cook, 1980). It has been shown that weed life cycle models have foundered on their sensitivity to predictions of weed seed to seedling dynamics (van der Weide & Groenendael, 1990; Freckleton & Watkinson, 1998). This may be explained by either a lack of relevant autecological data for individual weed species, or by failures in our understanding of the biological processes concerned. Moreover it is notable that the most successful predictions of weed seedling emergence to date, have been made for weed species that are relatively well understood, using explicit models of the biological processes involved (Vleeshouwers, 1997). These models have largely concentrated on

summer annual weed species and on the environmental controls of the processes of seed dormancy and seed germination. Summer annual species germinate exclusively in the spring, in contrast to the two winter annual species studied in this thesis. Winter annual species germinate mainly in the autumn, but also to a lesser degree in the spring. As such different environmental controls of seed dormancy and seed germination are required.

The aim of these next sections is to give a general introduction to seed dormancy and seed germination. The intention is to set this thesis in a broad context, clearly defining the biological processes involved and briefly outlining the environmental factors that affect seed dormancy and germination. This thesis subsequently identifies (Chapter 4) the most important of these factors for further consideration in developing quantitative models of these processes.

1.3. Seed dormancy

1.3.1. Definition and classification of seed dormancy

Dormancy has traditionally been identified as the absence of a germination response and accordingly, dormancy has often been defined as:

'*The temporary failure of a viable seed to germinate after a specified length of time in a particular set of conditions that later evoke germination when the restrictive state has been terminated by either natural or artificial means.*'
(Simpson, 1990 in Vleeshouwers, Bouwmeester & Karssen, 1995).

Whilst this definition has its uses, it cannot be applied uniformly to the phenomenon of dormancy. This is because there are several different types of dormancy, each with different causes, as shown in Table 1.1, as adapted from Baskin & Baskin (1989a).

Type	Cause(s) of dormancy	Characteristic of embryo
Physiological	Physiological inhibiting mechanism of germination in the embryo	Fully developed
Physical	Impermeable seed coat	Fully developed
Combinational	Impermeable seed coat; physiological inhibiting mechanism of germination in the embryo	Fully developed
Morphological	Underdeveloped embryo	Underdeveloped
Morphophysiological	Underdeveloped embryo; physiological inhibiting mechanism of germination in the embryo	Underdeveloped

Table 1.1: Types, causes and characteristics of seed dormancy.
(Adapted from Baskin & Baskin, 1989a).

Traditionally, dormancy has been viewed as an 'all or nothing' property, whereby seeds are either classed as dormant or non-dormant in a given set of environmental conditions. However it is clear from recent literature, notably Vleeshouwers *et al.* (1995), that whilst this might describe what has been termed physical and morphological dormancy, this definition is inappropriate for the more complex phenomenon of physiological dormancy. Before describing current advances in our understanding of physiological dormancy, physical and morphological dormancy will firstly be described in more detail.

1.3.2. Physical dormancy

As shown in Table 1.1, physical dormancy is caused by the impermeability of the seed coat. This type of dormancy has also been termed exogenous dormancy and it is typical of a number of plant families (notably the Fabaceae, Malvaceae and Chenopodiaceae (Copeland & McDonald, 1995)). It is largely genetically determined, but can be affected by the maternal environment during seed maturation (Karssen, 1970, in Bouwmeester & Karssen, 1993a).

It has been shown that the impermeability of the seed coat prevents water uptake by the seed and diffusion of oxygen to the embryo (Bewley & Black, 1994). There is also the suggestion that dormancy can be attributed to physical restraint on the growing embryo, such that the

embryo fails to rupture the seed coat (Baskin & Baskin, 1998). Evidence for this last mechanism is limited because of difficulties in controlling for other factors that may promote germination in the event of seed coat removal (Copeland & McDonald, 1995).

Physical dormancy is attributed to both physical and chemical attributes of the seed coat and under natural conditions is broken by either freeze-thawing cycles, ingestion by animals, micro-organism activity, fire and other factors (Copeland & McDonald, 1995; Bewley & Black, 1994). The effect of all these factors is to disrupt the integrity of seed coat and this process may take several years to complete. In the laboratory, physical dormancy can be broken by either mechanical or chemical scarification, with care taken to avoid seed damage (Hendry & Grime, 1993).

1.3.3. Morphological dormancy

Seeds that are shed before they are morphologically mature exhibit morphological dormancy. Dormancy results from the inability of the immature embryo to germinate (Baskin & Baskin, 1989a). Further embryo maturation occurs following seed dispersal and this may take only a few days or several months. The changes that occur in seeds after dispersal are generally known as 'after ripening'.

1.3.4. Physiological dormancy

Whereas seed with physical or morphological dormancy are clearly either dormant or non-dormant, physiological dormancy is defined on a continuous scale, whereby the wider the range of conditions at which a seed is able to germinate, the smaller its degree of dormancy. Defining dormancy as a continuous seed character allows a distinction to be drawn between the different causes by which a seed is unable to germinate, separating those causes related to dormancy from those related to germination (Vleeshouwers *et al.*, 1995). This has led to greater understanding of the mechanisms underpinning physiological seed dormancy and the different environmental factors involved in promoting germination. A good example of this is the work of Bouwmeester & Karssen (1992) which showed seasonal changes in the width of the temperature range over which exhumed seed of *Polygonum persicaria* germinated (i.e. seasonal changes in dormancy). These changes in dormancy were shown to be correlated with soil temperature, but not with seasonal changes in soil moisture, soil nitrate content or with the presence or absence of light, although these factors were shown to have an important influence on germination.

The importance of temperature in promoting changes in seed dormancy has also been shown in other studies of annual weed species including *Chenopodium album* (Bouwmeester & Karssen, 1993a), *Sisymbrium officinale* (Bouwmeester & Karssen, 1993b), *Spergula arvensis* (Bouwmeester & Karssen, 1993c), *Thlaspi arvense* (Baskin & Baskin, 1989b), *Lamium amplexicaule* and *Lamium purpureum* (Baskin, Baskin & Parr, 1986) and *Viola arvensis* (Baskin & Baskin, 1995). This is reviewed more thoroughly in Chapters 5 & 11, but it is clear that the effect of these temperature-mediated changes in dormancy is that germination tends to occur in seasons with conditions favourable for growth and reproduction (Baskin & Baskin, 1998). This allows seeds to survive periods of unfavourable conditions and more importantly, avoid germinating during short periods of favourable conditions at unfavourable times of the year (Vleeshouwers *et al.* 1995). This may largely explain the characteristic patterns of seasonal seedling emergence for many weed species (Roberts, 1964; Roberts & Feast, 1973; Roberts & Bodrell, 1983; Roberts & Neilson, 1980; Chancellor, 1986).

The study of seasonal changes in seed dormancy and dormancy in general is complicated for three main reasons. Firstly because it is, as yet, impossible to measure the state of dormancy of a seed and therefore dormancy has to be equated with an absence of germination (Vleeshouwers *et al.* 1995). This is confounded because temperature has a dual role in regulating both dormancy and germination (Probert, 1992). Finally although temperature has been shown to play an important role in dormancy regulation, the process is undoubtedly complex and almost certainly involves a number of different reactions which may be promoted or inhibited by plant growth regulators (e.g. abscisic acid) and other factors (Copeland & McDonald, 1995).

It is easier to study and understand the effect different environmental factors have on weed survival through the seedbank stage if a clear distinction is made between the phenomena of dormancy (in all its forms) and germination. The next section outlines the effect of temperature and other environmental factors on germination and describes the various methods used to study germination and assess seed viability.

1.4. Seed germination

1.4.1. Definition and measurement of seed germination

There are several different definitions of seed germination and it is important to understand their distinctions. Germination is variously defined as:

- the emergence of the radicle through the seed coat;
- 'the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions' (AOSA, 1991, in Copeland & McDonald, 1995)
- the resumption of active growth of the embryo resulting in the rupture of the seed coat and the emergence of the young plant (Bewley & Black, 1994).

The third definition presumes that the seed has been in a state of quiescence (or rest) following its formation and development and that it remains in this state until environmental conditions trigger a resumption in growth. From the previous discussion of the need to separate dormancy from germination, the validity of this definition is questionable. Between the other two definitions, there is essentially a difference in viewpoint and academic interest. The first tends to be the definition preferred by seed physiologists and the second by seed analysts (Copeland & McDonald, 1995). However, it should be noted that all three definitions are essentially flawed because the start of germination cannot be measured directly.

Measurements can accurately only be made at the end-point when either the radicle emerges or when a sustained increase in fresh weight begins. Given that the embryonic radicle may have grown prior to penetrating the surrounding tissues (and emerging) and because it is difficult to identify the precise point at which fresh weight starts to accumulate, these measurements may over-estimate the actual time taken to germinate by including some measure of seedling development. However this may not be important in studies comparing the percentage of seeds that germinate according to a particular treatment. It should be noted that measurement of fresh weight has the advantage of identifying seeds that have started to germinate, but failed to achieve radicle emergence. This is a phenomenon recognised by Bewley & Black. (1994), although there is no supporting evidence to indicate the extent to which this is actually a significant factor determining seed mortality in the field. However, it should also be considered whether the mechanics of measuring seed fresh weight interfere with the process of germination, which could then lead to anomalous results.

The timing of germination is an important characteristic of a seed population (Benjamin, 1990; Ghersa & Holt, 1995; Fenner, 1995). This is because the time of germination is correlated with the time of seedling emergence and this has been shown to be important in determining individual growth and development, especially in competitive environments. For this reason, it is important to emphasise that in efforts to improve predictions of weed seedling emergence, we need to be concerned not only with how many seeds germinate, but when and how this relates to prevailing environmental conditions.

1.5. Seed germination requirements

1.5.1. Water

Water is a basic requirement for germination (Mayer & Poljakoff-Mayber, 1975; Copeland & McDonald, 1995). At dispersal, seeds typically have a low water content with a relatively inactive metabolism (Bewley & Black, 1994). As such, seeds are said to be quiescent (or resting), though not necessarily dormant. Germination begins with water uptake by the seed (imbibition) and this starts a complex series of events including protein hydration, subcellular structural changes, macromolecular synthesis and cell elongation (Bewley & Black, 1994) which result ultimately in growth. The rate of imbibition is important. If it is too rapid, the seeds will be damaged (especially at low temperatures) but if it is too slow, the seedlings will be slow to emerge and at a competitive disadvantage (Bradford, 1995). The rate of imbibition is determined by seed coat permeability, seed-substrate contact and the soil water content (Bradford, 1995).

Soil water availability varies diurnally, seasonally and with depth in the soil profile. Diurnal variation in water availability is especially marked at the soil surface and seeds can be exposed to repeated dehydration - hydration cycles (Egley, 1995). Some seeds can tolerate desiccation for a short period after embryo growth has begun, but in most situations the embryo will be damaged and unable to resume growth (Baskin & Baskin, 1998)

As a general rule, seed germination is optimised at field capacity (Copeland & McDonald, 1995), i.e. the upper limit of the water-holding capacity of freely drained soils (Begon, Harper & Townsend, 1990). However it has been shown that some species can germinate if water potentials are reduced as low as −1.5 MPa (Bradford, 1995). In contrast, high soil water content has been shown to inhibit germination in some crop plants, including sugar

beet, corn and dwarf beans (Copeland & McDonald, 1995), although this has not been explicitly described in weeds and other non-crop plants. The inhibitory effect of high soil water content is thought to relate to the consequent reduction in soil aeration and availability of oxygen (Mayer & Poljakoff-Mayber, 1975).

1.5.2. Oxygen

Oxygen is required for the germination of most species (Karssen & Hilhorst, 1992; Kigel & Galili, 1995). The germination of some species, for example *Xanthium* sp. can also be promoted by oxygen concentrations higher than those of ambient air (Mayer & Poljakoff-Mayber, 1975). Respiration (an oxidative process) increases sharply with the onset of seed germination and if the oxygen concentration is reduced markedly below that of air, seed germination tends to be retarded (Bewley & Black, 1994). This has been shown for a large number of crop plants (Copeland & McDonald, 1995) and also for a number of weed species (e.g. Mullerverstedt, 1963 in Sobey, 1981). However this research has almost exclusively been conducted under laboratory conditions. The extent to which germination is inhibited by low partial pressures of oxygen in reasonably drained arable fields is probably limited as measured values in soil pores only exceptionally fall below 18 or 19 % O_2 (Harper, 1977; Karssen & Hilhorst, 1992). Low partial pressure of oxygen may occur in microsites such as those adjacent to plant roots or decaying organic matter and in soils in flooded areas (Karssen & Hilhorst, 1992).

Carbon dioxide tends to have the opposite effect on seed germination (Kigel & Galili, 1995). Increasing carbon dioxide concentrations to between 15 and 20 % partial pressure of air leads to many species failing to germinate (Karssen & Hilhorst, 1992; Ziska & Bunce, 1993), though the significance of these results is limited as carbon dioxide levels in the field rarely exceed 1 % (Karssen & Hilhorst, 1992). Decreasing the proportion of carbon dioxide supplied during germination does not tend to limit germination, although there are some species identified with a minimum carbon dioxide requirement for germination (Copeland & McDonald, 1995).

1.5.3. Temperature

The need to distinguish between the temperature requirements of the germination process as distinct from dormancy has already been described. Germination is complex process of different reactions, each of which is affected by temperature. For most species the

temperature optimum for germination lies between 15 and 30 °C, although germination may occur sub-optimally at temperatures above and below this range (Copeland & McDonald, 1995). Low temperatures and frost may cause significant injury to seeds, especially pre-dispersal resulting from the formation of ice crystals in the inter- and intra-cellular spaces (Bewley & Black, 1994). The extent of the damage varies between species and varies according to the temperatures involved; the duration of exposure; the moisture content of the seed and physiological maturity (Copeland & McDonald, 1995). Of these the moisture content of the seed is considered most critical (Copeland & McDonald, 1995), which in part may explain the reduction in frost sensitivity with seed age and reduced moisture content.

Responses to temperature are often expressed in terms of cardinal temperatures, that is the minimum, maximum and optimum temperatures at which germination occurs (Bewley & Black, 1994). A minimum temperature can be difficult to define because germination can be proceeding at too slow a rate to detect in the time allowed. The optimum temperature is defined as the temperature at which percentage germination is maximised within the shortest time period and the maximum temperature is determined by the temperature above which proteins and enzymes essential for germination are denatured. This temperature is rarely greater than 40 °C (Mayer & Poljakoff-Mayber, 1975). These cardinal temperatures can be identified for the whole process of germination (from imbibition to radicle emergence), or determined for each individual reaction or sub-process occurring as part of the process of seed germination. Within the optimal range of temperatures for germination, differences in response cannot be measured in terms of percentage germination, only in changes to the rate of germination.

The concept of thermal time gives some insight into the relation between germination rate and temperature. This is reviewed more thoroughly in Chapters 6 & 7, but is essentially a method to predict the time course of cumulative germination in seeds as a function of time and temperature (Benech-Arnold & Sanchez, 1995). This approach has been successfully used to demonstrate germination, temperature and time relations in controlled experiments. Further research is required to prove its usefulness in field conditions (with variable thermal environments), especially as temperature requirements may vary within and between populations depending on seed age and seed quality (Copeland & McDonald, 1995; Mayer & Poljakoff-Mayber, 1975). Moreover it is clear that germination in some species requires daily cycles of alternating (or fluctuating) temperatures (Thompson & Grime, 1983).

1.5.3.1. Alternating temperatures

Germination requirements for alternating temperatures are thought to be another important adaptation to restrict seed germination to conditions that increase the chance of seedling survival. For seeds on the soil surface, large diurnal temperature fluctuations indicate the absence of existing vegetation, which would otherwise insulate the soil surface. Similarly for seed incorporated into the soil seedbank, diurnal cycles of alternating temperatures are a means of sensing depth from the soil surface, with the width of the temperature fluctuations decreasing with greater depth in the soil profile (Thompson, Grime & Mason, 1977). Consequently germination requirements for alternating temperatures allow seeds to germinate on or near the soil surface in the absence of surrounding vegetation. It is generally considered that it is the range of temperature variation and not the actual temperatures that are important (Murdoch, Roberts & Goedert, 1989, Probert, 1992). However, the mechanism by which alternating temperatures are effective in promoting germination is unknown.

1.5.4. Light

Whilst water, oxygen and favourable temperatures are required for the germination of all seeds, not all species have a germination requirement for light. Some species germinate equally well in both light and darkness (possibly through the existence of a polymorphism for light requirements (Jones, 1992)), whilst the germination of others is inhibited by light. In a comparative study of the local flora of the Sheffield area (Grime *et al.*, 1981) showed that 104 of the 271 species tested achieved less than 10 % germination in the dark. As light rarely penetrates further than 5-10 mm into soil (Egley, 1995), a light requirement for germination means that only seeds at or near the soil surface will germinate (Kigel & Galili, 1995; Kryger Jensen, 1995). It is no coincidence that most light requiring seeds are small and unable to emerge from deep in the soil profile. As a light requirement prevents fatal germination, it acts as a depth sensing mechanism (Pons, 1992).

Identification of germination requirements for light is complicated by the fact that different aspects of the light climate have different effects on seeds, such as photon flux density (PFD), spectral composition and duration of exposure (Pons, 1992). Pons (1992) identifies two main types of light response:

- Effects of short duration exposure where response depends on wavelength and is largely independent of photon flux density above a threshold value.

- Effects of long duration exposures where response depends on both spectral composition and photon flux density.

In the field, short duration light exposure (less than 1 hour) occurs when the soil is disturbed, typically during cultivations (Froud-Williams, Chancellor & Drennan, 1983). Seed is brought to the surface and subsequently reburied. An existing leaf canopy will reduce PFD at all wavelengths relative to full daylight, but more in the photosynthetically active part of the spectrum (400-700 nm) than in the near infra-red (700-1000 nm) because of strong absorption by chlorophyll (Pons, 1992). This means that light beneath a leaf canopy is relatively depleted in the shorter red (R) wavelengths compared to the longer far-red (FR) wavelengths. This is significant because seeds are sensitive to different light wavelengths. Red wavelengths (660 – 700 nm) tend to be most effective in promoting germination and germination is inhibited by wavelengths in the far-red (730 nm), blue (440 nm) and below 290 nm (Mayer & Poljakoff-Mayber, 1975).

The phenomenon of photoreversibility of germination was first reported in 1952, following a series of experiments with lettuce seeds (Toole *et al.*, 1953 in Copeland & McDonald, 1995). Imbibed seeds were alternately exposed to red and far-red light and germination was alternately promoted and inhibited. This same response to light wavelength has since been demonstrated in a large number of seeds requiring light for germination and a mechanism involving phytochrome has been described (Bewley & Black, 1994).

Phytochrome is a generic name for a group of chromo-proteins that act as light receptors (Bewley & Black, 1994). Phytochrome can exist in one of two photo-interconvertible forms: a red light absorbing form (Pr) that has an absorption maximum in the red (660 nm) and a far-red absorbing form (Pfr) with an absorption maximum in the far-red (730 nm). Absorption of light by the Pr form converts it to Pfr, while light absorption by Pfr converts it back to Pr. Therefore red light tends to convert most of the phytochrome to the Pfr form and as red light promotes germination, germination would seem to be promoted by the existence of Pfr. The ecological importance of this system is that it allows a further sensing of whether the environmental conditions are suited to germination. If the short light exposure is depleted in the shorter red wavelengths, the red/far red ratio is decreased and the active Pfr is converted to the germination inhibiting Pr form of phytochrome. The PFD threshold for this reaction to occur is typically about 100 μmol m^{-2} s^{-1} (Pons, 1992), though germination has been promoted by photon fluxes as low as 3.7 μmol m^{-2} s^{-1} (Baskin & Baskin, 1979), 2

$\mu mol\ m^{-2}\ s^{-1}$ (Copeland & McDonald, 1995) and 0.026 $\mu mol\ m^{-2}\ s^{-1}$ (Bliss & Smith, 1985). It should be noted that the sensitivity of some seeds to such low photon fluxes has been implicated in promoting seed germination in dark treatments where green 'safe' lights have been used to record germination time courses (Baskin & Baskin, 1979; Grime *et al.*, 1981).

There are a number of species that require long exposure time for germination, for example *Plantago minor* (Pons, 1992). However for most species, long duration light exposure tends to inhibit germination, especially at high photosynthetically active photon flux densities (Hendry & Grime, 1993) and at low R:FR ratios (Pons, 1992). This may account for inhibition of germination at the soil surface (Bliss & Smith, 1985).

Light requirements (or lack of), in addition to varying between species, may vary with seasonal changes in seed dormancy (Milberg & Andersson, 1997). In a study of eight annual weed species, Milberg & Andersson (1997) showed that a light requirement for germination was induced in all eight species by burial. This is a common feature among weed species (Wesson & Wareing, 1969a, b; Froud-Williams, Drennan & Chancellor, 1984) and viewed as an important factor in facilitating the build up of persistent soil seedbanks (Grime *et al.*, 1981; Pons, 1992). However Milberg & Andersson (1997) additionally demonstrated seasonal variability in germination response to light for five of the eight species. This again may be an adaptation to prevent seeds germinating during short periods of favourable conditions at unfavourable times of the year. Other authors have shown that light requirements and temperature may interact with seed germination dependent on light at one temperature and not at others (Pons, 1992), but this is not always the case (Goudey, Saini & Spencer, 1988). Additionally light can often substitute for a germination requirement for alternating temperatures (Pons, 1992)

Seed light responses may interact with water potential and length of the imbibition period. Pons (1992) states that there is a requirement for higher R:FR ratios at low water potential to promote germination and Copeland & McDonald (1995) state that typically the light requirement for germination is progressively reduced with increasing time from imbibition (Copeland & McDonald, 1995).

For a number of species, light and nitrate ions have been shown to interact positively to promote germination (Vincent & Roberts, 1977; Roberts & Benjamin, 1979; McGinley, Temme & Geber, 1987; Goudey, Saini & Spencer, 1988). In some studies this interaction

has been shown to depend on Pfr (red light) (McGinley, Temme & Geber, 1987) whilst in others, an inter-dependence of the interaction with temperature (Vincent & Roberts, 1977; Roberts & Benjamin, 1979).

Several other chemicals have also been shown to either inhibit or promote germination. The following description will be limited to those that naturally occur within seeds or that are commonly found in the soil.

1.5.5. Abscisic acid (ABA)

Abscisic acid (ABA) is an endogenous plant growth regulator that can act as a germination inhibitor. In some species the extent of seed dormancy is correlated with the concentration of ABA in the seed embryo (Mayer & Poljakoff-Mayber, 1975) and in fruiting species, the concentration of ABA within the fruit is high in order to prevent premature germination and vivipary (Karssen & Hilhorst, 1992). Experiments with exogenous application of ABA have shown that germination can be inhibited and that subsequent removal of ABA allowed seed germination (Mayer & Poljakoff-Mayber, 1975). However an explicit role of ABA in dormancy induction has not been demonstrated, as levels of ABA do not necessarily decline during germination (Mayer & Poljakoff-Mayber, 1975). Instead it is thought likely that ABA interacts with changing concentration of other plant growth regulators which act as germination promoters (Mayer & Poljakoff-Mayber, 1975).

1.5.6. Gibberellins

Gibberellins are a group of endogenous plant growth regulators known to promote seed germination of a large number of species, substituting for light and specific temperature requirements (Copeland & McDonald, 1995; Mayer & Poljakoff-Mayber, 1975). Natural gibberellin-like substances have been shown to occur in seed during particular stages of after-ripening and germination, suggesting that gibberellins play a role in the regulation of seed germination (Bewley & Black, 1994).

1.5.7. Cytokinins

Cytokinins (another group of endogenous plant growth regulators, including kinetin) have also been shown to promote seed germination, substituting for light and chilling requirements (Copeland & McDonald, 1995). The mechanism by which cytokinins affect

seed germination is similarly unknown, but several suggestions have been made including regulation of gene expression, promotion of enzyme synthesis and mediation of membrane permeability (Bewley & Black, 1994).

1.5.8. Auxins

Auxins are another group of endogenous plant growth regulators. Auxins have been shown to effect seed germination, but the effects have not been consistent, such that Copeland & McDonald (1995) draw the conclusion that generally auxins have been shown to have little effect on promoting seed germination. That said, exogenous application of indolacetic acid (IAA) has been shown to increase lettuce seed germination and there is evidence to suggest an interaction between IAA and light on seed germination (Mayer & Poljakoff-Mayber, 1975). The effect is thought to be dependent on the concentration of IAA used, with high concentrations tending to inhibit germination (Mayer & Poljakoff-Mayber, 1975).

1.5.9. Ethylene

Ethylene (C_2H_4) is a recognised plant growth regulator that has further been shown to either stimulate or inhibit germination (regardless of seed age) and is known to be released during the germination of several species (Egley, 1995; Copeland & McDonald, 1995). Ethylene is also a common constituent of the soil atmosphere (Karssen & Hilhorst, 1992). It is thought that ethylene is involved in regulating the level of auxins (see section 1.5.8) in seed and has been shown to interact both with gibberellins and red light to promote the germination of lettuce seeds (Copeland & McDonald, 1995). There is also a suggestion that ethylene interacts with nitrate to stimulate germination (Saini, Bassi & Spencer, 1985a, b).

1.5.10. Nitrates

Nitrate is the major naturally occurring inorganic soil component that stimulates seed germination (Karssen & Hilhorst, 1992). Numerous studies have shown this effect and in seed testing, potassium nitrate (KNO_3) is the chemical most commonly used to promote seed germination (Bewley & Black, 1994). In general germination is stimulated within a range of 0 to 50 mmol l^{-1} nitrate and germination is inhibited by supra-optimal concentrations (Karssen & Hilhorst, 1992). Soil nitrate concentrations tend to correspond to this optimal range, though there are substantial variations according to changing temperatures and soil water potentials. Accordingly the effect of nitrate on seed germination cannot be considered

in isolation from these and other biotic factors. Of these, light is particularly important as discussed in section 1.5.4. As regards the mechanism of nitrates in stimulating germination, it has been variously suggested that nitrates act to increase oxygen uptake by the germinating seed or act as a co-factor for phytochrome (Bewley & Black, 1994).

1.5.11. Other chemicals in the soil

Other species-specific chemicals that affect germination have been identified, including allelopathic chemicals that either inhibit or delay germination of seed of different species and chemicals released that promote the germination of neighbouring seeds of the same species (Mallik, Puchala & Grosz, 1994).

1.5.12. pH

Seeds of most species can germinate readily across a wide range of hydrogen ion concentrations, from pH values of 4.0 to 7.6 (Mayer & Poljakoff-Mayber, 1975). Within that range, different species may have specific optima.

1.5.13. Mechanical damage

Mechanical injury, damaging the seed coat or internal seed structure is thought to be a bigger problem in large-seeded species. The damage can have direct effects on seed viability or indirect effects via increased susceptibility to micro-organisms.

1.5.14. Ionising radiation

From a number of reports, it has been shown that seed germination is retarded (but not inhibited) by exposure to gamma radiation (Copeland & McDonald, 1995).

Before going on to identify the aims and objectives of this thesis and give an outline structure, it is important to briefly explain why *Stellaria media* and *Galium aparine* were selected for this study into the effects of different environmental and management factors on seed dormancy and germination in winter annual weed species.

1.6. Species selection

The focus on winter annual weed species reflects the relative importance of winter cropping in the UK and Scotland (Cousens & Mortimer, 1995). The selection of *S. media* and *G. aparine* in particular reflects the importance of these two species in the broad-leaved weed vegetation of temperate winter crops (Attwood, 1985).

These two species also differ markedly in key characteristics such as seed size, seed persistence, the growth habits of seedlings and longevity of plants and it was considered that study of these contrasting weed species could allow generalisation or suggest hypotheses for differences in seed dormancy and germination requirements. However it was noted that this approach ignores the arguments in the ecological literature that the value of comparative studies is maximised by studying closely related species (Harvey, Read & Nee, 1995a, b; Westoby, Leishman & Lord, 1995a, b). General characteristics of the two species are summarised below:

1.6.1. Stellaria media

S. media is the most common broad-leaved weed of arable crops in the United Kingdom (Tottman & Wilson, 1990). It thrives in cool, wet conditions and on fertile soils (Sobey, 1981). It tolerates low temperatures and readily over-winters to produce large competitive plants in early spring (Tottman & Wilson, 1990). Germination can occur throughout the year, but has distinct peaks in the spring and autumn (Sobey, 1981). Large numbers of seeds are produced by mature plants and dormancy is very variable (van der Vegte, 1978; Sobey, 1981). Some seeds germinate immediately after shedding, whilst others join a persistent seedbank in the soil (Sobey, 1981). The variable germination and short life cycle make this a very successful weed species.

1.6.2. Galium aparine

This is the most aggressive weed of winter cereals in the United Kingdom (Tottman & Wilson, 1990). Its climbing and scrambling habit allows even small populations to form a dense canopy over the crop causing severe lodging and loss of yield, such that 20 - 30 plants m^{-2} can halve wheat yield (Tottman & Wilson, 1990). Seeds are difficult to separate from wheat grain and are often spread by drilling contaminated seeds (Tottman & Wilson, 1990). Germination requires fairly low temperatures and therefore occurs mainly in the autumn and

early winter (Malik & van den Born, 1988). Dense infestations can result in the production of over 20,000 viable seeds m^{-2} and individual plants at low densities can produce over 1,000 seeds per plant (Tottman & Wilson, 1990). Dormant seeds can persist in the soil for at least 18 months (Malik & van den Born, 1988). Seeds are shed late in the season and most are still attached to plants at the time that crops are harvested.

1.7. Aims and objectives

The aim of this thesis was to better understand the ecology of two common, winter annual weed species, *Stellaria media* and *Galium aparine*.

This aim was founded on the need to reduce reliance on herbicides and promote alternative methods of weed control in arable cropping. It is argued that these more targeted and sustainable methods of weed control would benefit from predictions of the timing and extent of weed seedling emergence. Ecological investigations were therefore focused on the sub-processes of seed dormancy and seed germination. These processes were selected as they were thought to represent the greatest source of uncertainty in predicting weed seedling emergence.

The thesis hinges on a modelling approach to direct investigations and summarise findings through the development of models of seed dormancy and germination for *S. media* and *G. aparine*. The key benefits of this approach are that it clearly identifies where current understanding is limited and may ultimately offer the prospect of quantitative predictions of weed seedling emergence.

These two winter annual species were selected because of their relative importance in the weed vegetation of British winter crops, and their contrasting ecological characteristics (as described in section 1.6). It was hoped that investigating the range of behaviour associated with these two different species would identify general factors important in modelling patterns of seed dormancy and seed germination in winter annual species.

A central theme of this thesis was the extent of intraspecific variability and how this relates to efforts at modelling weed seed germination. Large-scale intraspecific variation has been demonstrated for many weed species, but existing models of weed seed germination do not account for this variation. This variation has both a genetic and environmental basis, and it

is expressed as a complex interaction between individual genetic structure, prior history (maternal effects) and prevailing local conditions. In order to address the issue of whether this variation was important in modelling weed seed germination for *S. media* and *G. aparine*, efforts were made to dissect this variation. This meant that investigations to support model development were conducted with different populations of both species, each produced in a common environment (to limit maternal effects). It also meant that investigations were included to quantify the extent of variation between seed lots with different prior history (maternal environments), however, these studies were not incorporated into the final model.

Following from these needs, this thesis set out to assess the validity of the following hypotheses:

1) There are genetic (heritable) differences between populations of *S. media* and *G. aparine* in seed dormancy and germination characteristics (demonstrated by controlling maternal effects).

2) Differences in the maternal environment (in which seeds are produced) can account for significant variability in *S. media* and *G. aparine* germination characteristics within a population (demonstrated by controlling for genetic (heritable) effects).

3) There is an interaction between population genetics and conditions in the maternal environment that produces further variability in *S. media* and *G. aparine* seed dormancy and germination characteristics.

It also set out to address the following questions:

a) Can quantitative models of seed dormancy and germination be developed for *S. media* and *G. aparine* that account for variation between populations?

b) To what extent would such models need to be adapted to account for variation related to differences in maternal environments?

c) Can understanding of *S. media* and *G. aparine* seed responses to environmental conditions be usefully synthesised into quantitative models?

d) Can these models effectively describe the observed experimental patterns of *S. media* and *G. aparine* seed dormancy and seed germination or does this approach reveal flaws in understanding?

e) Can the resulting fitted model be a useful tool in predicting *S. media* and *G. aparine* seedling emergence in the field, or is it a useful tool for identifying future research needs?

1.8. Thesis outline

This thesis has two central themes in promoting better understanding of the ecology of *S. media* and *G. aparine*, namely variation and modelling. The theme of variation runs through the thesis, following on from an initial large-scale assessment of variation in germination and seedling growth characteristics of populations of *S. media* and *G. aparine*, collected from across the UK and Europe. This is described in Chapter 2 and Chapter 3 and addresses hypothesis (1) by documenting the extent of genetic variation between populations using seeds produced in a common environment. These chapters also aim to identify three populations for each species that differ markedly in seed germination and seedling growth characteristics. These selected populations were then used in subsequent investigations to assess in more detail, the variability of populations in response to different treatments, as determined by the modelling objective.

The thesis then considers variation between contrasting populations in the context of model development. Chapter 4 introduces the modelling theme, outlining the general principles of modelling and reviewing existing weed population models, particularly those geared at understanding weed seed dormancy and germination. This chapter then identifies the preferred modelling framework with which to address questions (a, c, d & e). It also reviews existing data related to seed dormancy and germination in *S. media* and *G. aparine* and identifies the main areas of weakness in current understanding. These deficiencies then direct subsequent investigations in order to support model development.

Chapter 5 presents results from a year-long burial experiment using seed produced in a common environment for the three contrasting populations of *S. media*. This was to counter limited understanding of seasonal dormancy changes in *S. media*.

Chapter 6 and Chapter 7 investigate the combined effects of constant temperatures and water potential on the timing and extent of seed germination in *S. media* and *G. aparine* respectively. Chapter 6 additionally considers the interactions with or without light. In both chapters comparisons are drawn between the three contrasting populations of each species, using seed produced in a common environment. Chapters 6 and 7 also investigate the application of existing hydrothermal time models of seed germination.

Chapters 8 and 9 summarise the effects of low dose herbicide application on seed production and seed germination characteristics for *S. media* and *G. aparine* respectively. Low dose herbicide applications were selected as an easy method of manipulating plant growth in the maternal environment and they also represent a more integrated approach to weed control in arable cropping. This addresses hypothesis (2) by moving the focus to consideration of maternal effects and hypothesis (3) by contrasting population response to maternal effects.

Chapter 10 quantifies the effects of nitrogen on seed germination for *S. media*. This is investigated both for the immediate effect of different nitrogen ions on seed germination and for the germination characteristics of seeds produced in soils differing in nitrogen status. This thereby considers both population genetic differences and interaction with differences related to the maternal environment (hypotheses 2 and 3).

Chapter 11 addresses the need to synthesise the data collected by formulation of new quantitative models of seed dormancy and germination for *S. media* and *G. aparine*. This addresses questions (a, c & d) whilst questions (b & e) are addressed by discussion of model application.

Chapter 12 is the final discussion and summary of the main results, with identification of future research priorities.

This thesis addresses important deficiencies in understanding of *S. media* and *G. aparine* dormancy and germination. It also attempts to synthesise seed responses to environmental conditions in a quantitative model, with relatively simple formulation and potential for further development. It is hoped that the improved understanding contributed by this study can lead to the development of more environmentally sensitive weed control methodologies.

Chapter 2. The selection of contrasting populations of *Stellaria media* (L.) Vill.

2.1. Summary

Seeds from 25 populations of *Stellaria media* (L.) Vill. were collected from arable field sites in the United Kingdom and grown for a generation in a common environment. Second generation seed were then used to limit maternal sources of variation. Significant between population differences in physical seed characteristics, seed germination patterns and seedling growth statistics were recorded. These characteristics were used in a multivariate cluster analysis to select three contrasting populations to use in more detailed studies.

This chapter also compares two different methods for comparing seed germination time courses and uses partial least square regression analysis to identify possible physical predictors of seedling dry weight and seedling relative growth rate.

2.2. Introduction

It has been acknowledged that a major criticism of most studies of germination ecology is their failure to study more than one population. A central theme of this thesis is to identify the extent of variation in seed and seedling behaviour within and between populations of two contrasting winter annual weed species. It is hoped that better understanding of seed dormancy and seed germination might allow better prediction of patterns of seedling emergence and that this might lead to more targeted efforts at weed control by either cultural means or by appropriate low dose herbicide applications.

This chapter presents the results of a large scale screening of 25 populations of *S. media* to assess the variation between populations in physical seed characteristics, seed germination and seedling establishment and to select contrasting populations for the more detailed studies presented in subsequent chapters.

S. media is a widespread annual species that commonly occurs as a weed of arable crops and reseeded grassland. As a widespread species that tends to reproduce asexually (Sobey, 1981), it is likely that *S. media* populations differ markedly as a result of local selection

pressures. Differences in *S. media* germination ecology have previously been recorded for two populations from a field in the Netherlands (van der Vegte, 1978). The two populations differed markedly in the temperature range over which freshly collected seeds would germinate and whilst one population built up a relatively uniform short-lived seedbank, the other built up a persistent seedbank of phenotypically diverse seeds. Other examples of populations differentiation in the literature include differences in germination behaviour between *S. media* populations in response to temperature and water potential (Grundy, 1997) and differences between populations in susceptibility to sulfonylurea herbicides (Kudsk, Mathiassen & Cotterman, 1995).

As detailed above, differences between populations have been described previously. However there has not been a widescale survey of *S. media* in which variation between populations has been assessed on a common basis, using seeds from plants grown for a generation in a common environment. As explained in Chapter 1, this is considered important as differences in seed and seedling characteristics can be caused by differences in the maternal environment during seed formation. Producing seed in a common environment is considered to reduce these maternal effects and allow a comparison of populations that better reflects genetic differences between populations (Quinn & Colosi, 1977; Roach & Wulff, 1987).

2.3. Methods

2.3.1. Seed collection

Seeds from field populations of *S. media* were collected at maturity from 25 arable sites across the United Kingdom over the summer of 1995. Seeds were collected from a large number of plants at each site and were allowed to dry at room temperature for approximately two weeks. Cleaned seeds were then transferred (in paper envelopes) to an incubator maintained at 10 °C (± 2 °C). A tray of silica gel was placed within the incubator to reduce humidity. Each seed collection was labelled with site details including field name, farm name and National Ordnance Survey (OS) grid reference and with the harvest date and name of collector. Additional information on cropping history was recorded where this was available.

2.3.2. Seed replication in a common environment

Seeds were multiplied in a common environment starting in October 1995. Twenty seeds were sown in three replicate pots for each population, which were randomly arranged in three blocks in an unheated greenhouse. Pots were watered regularly. In January 1996, the plants were transferred to a heated greenhouse (target temperature 10 °C) following exceptionally low temperatures in late December. Seedling emergence was recorded and the seedlings were thinned to 5 per pot. As the plants grew, canes and twine were used for support and to restrict interaction between neighbouring pots. Cross fertilisation between populations was prevented by covering the pots at the time of first flowering with a layer of muslin sealed at the bottom with an elastic band. Mature seed was collected over a period of 6 to 8 months, dried, cleaned and stored as described above. Seeds collected at different times were stored separately and only that collected in August 1996 was used in the screening experiment.

2.3.3. Screening for differences in germination characteristics

Starting in November 1996, the germination characteristics of the 25 populations were assessed in two controlled environment cabinets (FISONS), each maintained with a 14 hr day. The warm cabinet (W) was set to maintain 20 °C during the day and 15 °C at night and the cold cabinet (C) was set to10 °C during the day and 5 °C at night. The irradiance during the day varied between a photosynthetically active photon flux density (PPFD) of 160 to 205 μmol m^{-2} s^{-1} (as recorded using a quantum sensor and a Campbell CR10 datalogger). To counter the lack of replication of the controlled environments, replicates were started at approximately 10 day intervals. For each of the three replicates, 100 seeds were counted, weighed and placed on 2 layers of filter paper (Whatman Grade 181) in a 9 cm Petri-dish. The papers were then moistened with 10 ml of distilled water and placed in a randomised block on one shelf in the cabinet. The seeds were checked after 24 hrs, then 3 times daily for the next 4 days, (coinciding with peak of germination) followed by further daily checks for another 7 days. The Petri dishes were inspected in the same order on each occasion. The Petri dishes were removed and recorded at room temperature under fluorescent light. Seeds that had germinated (as defined by radicle emergence > 1 mm) were counted and removed.

For statistical analysis of germination time courses, two statistical models were used.

2.3.3.1. Analysis by fitting Gompertz curves to cumulated seed germination

The Gompertz curve is widely used to describe germination time courses (Brown & Mayer, 1988; Vleeshouwers, 1997). Gompertz curves were fitted to the cumulated seed germination data for each Petri dish by least squares (Genstat 5). The Gompertz curve relates the cumulative germination fraction, g to time, t as follows:

$$g = \alpha + \gamma \exp(-\exp(-\beta(t-\mu))) \qquad (2.1)$$

where the parameter β is related to the slope of the curve and larger values of β are associated with greater synchronicity of germination. The parameter μ equals the time from the start of the experiment to the point of inflexion in the curve and the parameters α and γ are the asymptotes of the fitted curve, the sum of which represents the total number of seeds germinated for each population. The Gompertz curve was also used to estimate time to 50 % germination (t_{50}) for each data set. Analysis of variance (Genstat 5) was used to identify differences in the fitted parameters of the Gompertz curve and estimated t_{50} between populations and between test environments, excluding data sets where the Gompertz curve was poorly fitted ($r^2 < 0.9$).

2.3.3.2. Analysis by a maximum likelihood approach

Maximum likelihood is a widely used statistical technique. In order to apply this method it is first necessary to specify a model that describes the distribution of the data. It was assumed that a fraction of the seeds will fail to germinate and the times to germination of the remainder were normally distributed (Hunter, Glasbey and Naylor, 1984). The model parameters estimated for each data set were consequently the mean time to germination (m), the variance of the time to germination (v) and the proportion of the seeds failing to germinate (p). It was also assumed that seeds germinate independently of each other. These assumptions enabled calculation of the likelihood, that is the probability that the specified model could have generated the observed data. Parameter estimates were then obtained by maximising the likelihood with respect to m, v and p. A useful property of this method is the ability to calculate standard errors for each estimated parameter. Details of this method are given in Hunter, Glasbey and Naylor (1984) and implementation used purpose written software including routines from the NAG library (Numerical Algorithms Group, 1999) with the program supplied by Dr. Glenn Marion. Analysis of variance (Genstat 5) was used to identify differences in the estimated model parameters between populations and between test

environments, excluding data sets where the model was poorly fitted (time to 75 % germination (t_{75}) > 1000 hours).

2.3.4. Screening for differences in seedling growth characteristics

A subset of the first seeds to germinate in the warm cabinet were transferred to a growth room maintained with the same temperature and day length conditions (14 hr day; 20/15°C). PPFD varied between 75 to 90 $\mu mol^{-1}\ m^{-2}\ s^{-1}$. A minimum of 40 germinated seeds per population (except Cambridge 1) were transferred and sown individually into 30 mm diameter cells in 10 x 15 cell-trays filled with potting mixture (John Innes no. 2). Seed diameter was measured to the nearest 0.05 mm at sowing, using callipers. Seed colour was recorded on an arbitrary scale of 1 to 3 (light brown to black). Seedling height and width were measured at emergence (cotyledons expanded) and at the two-leaf stage and the time taken from sowing to each growth stage for each individual was recorded. For each population, random samples of a third of the established seedlings were harvested at emergence, at two leaves and at four leaves. The dry weights of harvested seedlings were recorded and their relative growth rates calculated by subtracting the natural log of mean population seed weight from the natural log of each seedling dry weight and dividing by the difference in days to emergence and days to reach the particular growth stage (methods follow Hunt, 1978a, b). Analysis of variance (Genstat 5) was used to identify differences between populations for each of the seed and seedling characteristics, except seed colour which was assessed by contingency table.

2.3.5. Prediction of dry weight and relative growth rates from other seedling characteristics

The measurement of individual dry weights and the calculation of relative growth rates depend on destructive sampling of seedlings. Moreover *S. media* seedlings are small, with dry weights ranging from 0.5 mg at emergence to about 4 mg at the four leaf stage and therefore the measurement of individual dry weight for a large number of seedlings is tedious. In order to avoid destructive sampling, partial least squares regression analysis was used to assess whether other seed or seedling characteristics could be used to predict seedling dry weight or seedling relative growth rate.

Partial least squares regression (PLS) is a method of relating two multivariate data sets, that relates several dependent (Y) variables to several independent (X) variables. The method

aims to identify the underlying factors, or linear combinations of the independent variables, which best model the dependent variables. The method involves an iterative procedure in which firstly a linear combination of the independent variables is estimated to give a latent vector with the property that all the dependent variables can be predicted optimally from this vector by least squares. A second latent vector is then derived which represents a linear combination of the residuals of the independent variables after projection onto the first latent variable. This vector has the property of the optimally predicting the residuals of the dependent variable from the first stage. This procedure is continued until the contribution of a new latent vector is negligible. Details of the PLS algorithm are given in Aastveit & Martens (1986).

Cross validation was used to predict the optimal number of latent variable factors required for interpretation. This involved splitting the data into five groups and running the procedure repeatedly, omitting each group in turn and estimating factor scores from the reduced data. For each run, the fitted model was used to predict values for the missing observations and the sum of squares of differences between observed and predicted was calculated. The sums of squares of differences between all the observed and predicted values were then used to assess the predictive value of each factor.

The PLS method (Genstat 5, with program supplied by Dr. Mike Talbot) was applied to data in a 25 x 10 matrix of 10 independent (X) variables. These were seed diameter and colour, seedling height and width at emergence and two leaves; seedling height growth rate and time to emergence, two leaves and four leaves. The dependent (Y) variable formed a 25 x 5 matrix. The 5 dependent variables were seedling dry weight at emergence, two leaves and four leaves and seedling relative growth rate to two and to four leaves. The columns of both matrices were transformed to zero mean and unit variance before the analysis.

2.3.6. Selection of contrasting populations

In order to select contrasting populations for further study, a similarity matrix was constructed from the combined seed size, seed colour, seed germination and seedling growth statistics. The statistics entered are listed in Table 2.1 and were unweighted, except that seed germination data was represented by the two different methods of analysis. The similarity matrix was then entered into a furthest neighbour hierarchical cluster analysis (Genstat 5). The aim was to identify three populations, two representing populations with contrasting

behaviour and characteristics and one representing a 'typical' *S. media* population. This was done by identifying the lowest level at which three clusters were represented and selecting populations from within each cluster. The 'normal' population was selected from the largest cluster.

The results from cluster analysis were checked by ranking populations for each statistic and summing the number of top five and bottom five rankings with the highest scores identifying the two most 'extreme' populations and the lowest score identifying the most 'average' population.

Seed	Seed germination	Seedling growth
• 100 seed weight (g)	• Percentage germination	• Percentage emergence
• Seed diameter (mm)	• Gompertz parameter β	• Time from sowing to emergence
• Seed colour	• Gompertz parameter μ	• Time from sowing to two leaves
	• Gompertz parameter α	• Time from sowing to four leaves
	• Gompertz parameter γ	• Height at emergence
	• Time to 25 % germination	• Height at two leaves
	• Time to 50 % germination	• Width at emergence
	• Time to 75 % germination	• Width at two leaves
	• Max. likelihood mean (m)	• Dry weight at emergence
	• Max. likelihood variance (v)	• Dry weight at two leaves
	• Max. likelihood proportion germinated (p)	• Dry weight at four leaves
		• Relative growth rate from height at emergence to two leaves
		• Relative growth rate in dry weight from sowing to two leaves
		• Relative growth rate in dry weight from sowing to four leaves

Table 2.1: List of statistics entered into cluster analysis for selection of contrasting populations.
Data are population means.
Data for seed germination are given for both test environments.

2.4. Results

2.4.1. Sites

Table 2.2 shows the location and description of sites from which the 25 populations were collected. The sites ranged in latitude from 51° 0′ to 58° 5′ N. It should be noted that sites were biased to areas of arable cropping and that a relatively large number of sites in central Scotland were represented. It should also be noted that samples were largely taken from experimental farms, especially in England. All the sites were in arable rotations and seeds were sampled from a range of crops including winter wheat, winter barley, spring wheat, linseed, cabbages, broccoli, turnips and rotational set-aside. The sites ranged in elevation from 0 to 280 metres above mean sea level. The Borders, Perthshire and Stirlingshire sites bordered major rivers and the Benbecula site was < 500 m from the seacoast.

2.4.2. Seed replication

Figure 2.1 shows the variation in temperature during seed replication in a common environment. Mean daily temperature varied from – 4 to 25 °C.

Figure 2.2 shows the initial percentage seedling emergence for seeds sown out for replication in a common environment. The populations are presented according to latitude, in order from south to north. It is clear from Figure 2.2 that initial seedling emergence varied between populations, from 7 % for the Lothian 3 population (19) to 98 % for the Cheshire population (13). However, there was no apparent trend in seedling emergence according to latitude.

Figure 2.3 shows the number of days from sowing to seedling emergence for seeds sown out for replication in a common environment. The number of days between sowing and emergence varied between 7 and 17 days for different pots, with a mode of 8 days. Overall there was little difference between populations in terms of time from sowing to emergence, although it is interesting to note that the shortest time was recorded for the Cheshire population (13) and the longest time which was recorded for the Lothian 3 population (19). Again there were no apparent geographical patterns in time between sowing and emergence.

Figure 2.4 shows the number of days from sowing to first flowering of the different populations of *S. media* grown in a common environment. The number of days between

sowing and flowering varied between 97 and 190 days for different pots. A large number of populations flowered synchronously between 97 and 98 days after sowing, including all the populations sampled from central England. The populations from Lanarkshire (16) and Perthshire (23) required substantially longer time to flower and in the case of the Perthshire population only two out of the three replicates flowered. Again there was no apparent geographical pattern in time between sowing and flowering.

Code	Administrative County	Ordinance Survey Grid reference	Latitude	Longitude	Elevation (m) above sea level
1	Hampshire 1	SU 341 518	51° 04′ N	1° 19′ W	80-90
2	Hampshire 2	SU 525 337	51° 04′ N	1° 19′ W	100
3	Kent	TR 065 445	51° 11′ N	0° 56′ E	30-40
4	Hertfordshire	TL 106 133	51° 49′ N	0° 22′ W	110
5	Cambridgeshire 1	TL 345 645	52° 15′ N	0° 02′ W	40
6	Cambridgeshire 2	TF 441 824	52° 24′ N	0° 16′ E	0-10
7	Leicestershire	SK 798 026	52° 34′ N	0° 52′ W	180
8	Norfolk	TG 044 970	52° 34′ N	1° 07′ E	30-40
9	Herefordshire 1	SO 567 507	52° 09′ N	2° 41′ W	130-140
10	Herefordshire 2	SO 586 523	52° 09′ N	2° 41′ W	190-200
11	Warwickshire	SP 270 563	52° 19′ N	1° 35′ W	40-50
12	Nottinghamshire	SK 588 707	53° 13′ N	1° 08′ W	60-70
13	Cheshire	SD 656 860	53° 23′ N	2° 32′ W	50
14	North Yorkshire	TA 883 692	54° 08′ N	0° 48′ W	180
15	Borders	NT 625 313	55° 34′ N	2° 38′ W	70-80
16	Lanarkshire	NS 933 444	55° 40′ N	3° 46′ W	200-210
17	Lothian 1	NT 163 630	55° 53′ N	3° 20′ W	270-280
18	Lothian 2	NT 514 688	55° 55′ N	2° 48′ W	100-110
19	Lothian 3	NT 248 649	55° 52′ N	3° 12′ W	200-210
20	Fife	NO 324 084	56° 16′ N	3° 05′ W	60-70
21	Angus	NO 530 362	56° 31′ N	2° 46′ W	30-40
22	Stirlingshire	NO 830 215	56° 22′ N	3° 50′ W	50
23	Perthshire	NO 178 209	56° 23′ N	3° 20′ W	10
24	Benbecula	NF 765 521	57° 26′ N	7° 23′ W	0
25	Caithness	ND 280 606	58° 30′ N	3° 14′ W	30-40

Table 2.2: Locations and site descriptions for collected populations of *Stellaria media*

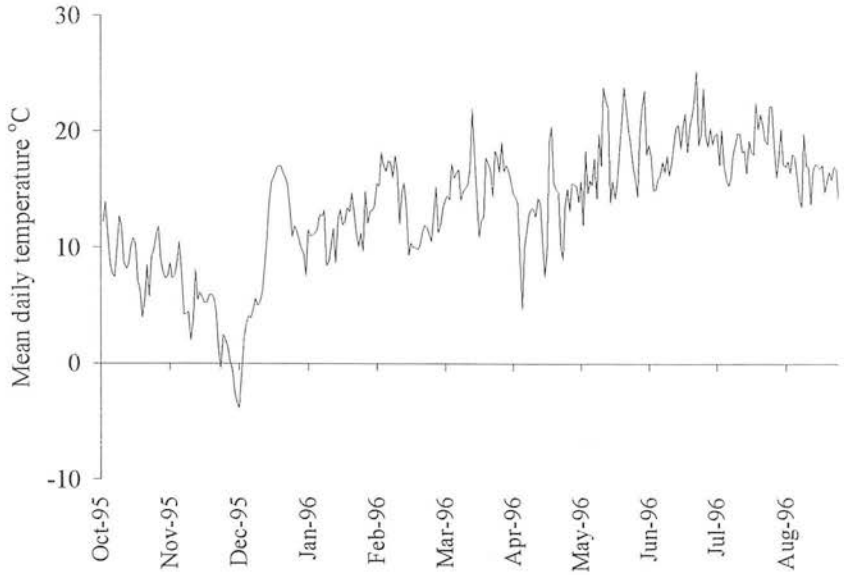


Figure 2.1: Temperature variation in common environment.
Data are the mean of air temperature recorded at 30 minute intervals.

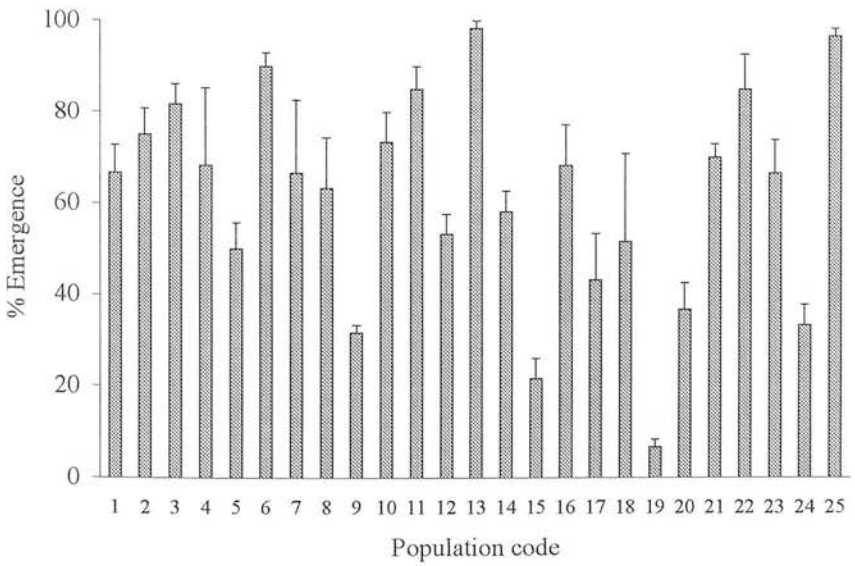


Figure 2.2: Initial percentage seedling emergence for seeds of *Stellaria media* sown out for replication in a common environment.
n = 3; 20 seeds sown per replicate; mean emergence ± 1 S.E.
Population codes as Table 2.2 and presented according to latitude, in order from north to south

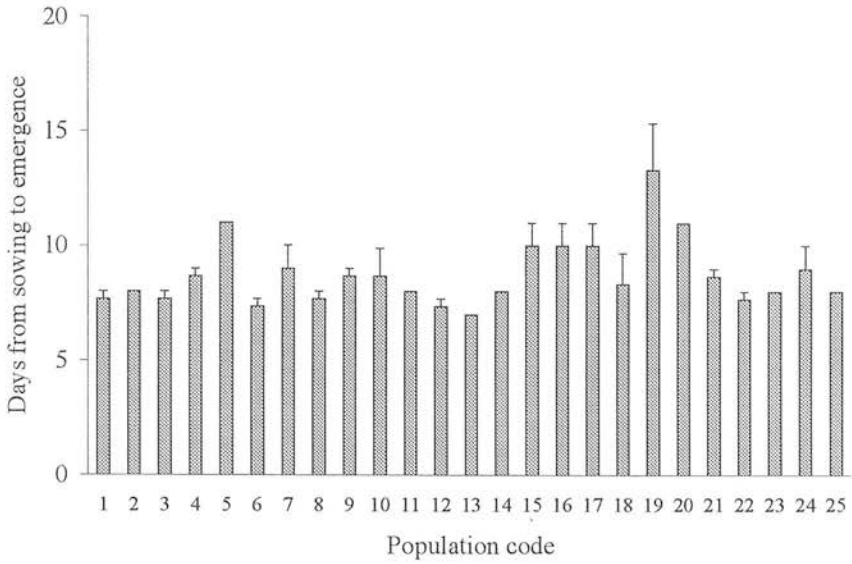


Figure 2.3: Days from sowing to first emergence for seeds of *Stellaria media* sown out for replication in a common environment.
Other details as for Figure 2.2.

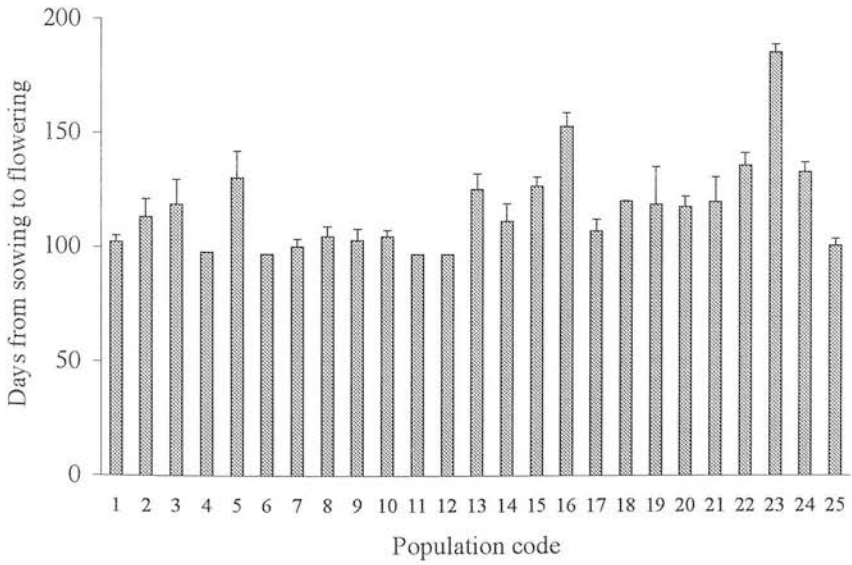


Figure 2.4: Days from sowing to first flowering for seeds of *Stellaria media* sown out for replication in a common environment.
Other details as for Figure 2.2.

2.4.3. Screening for differences in seed characteristics

Figure 2.5 shows differences between populations for mean 100 seed weight seed diameter. There were significant differences between populations with the heaviest seeds from Caithness (25) and the lightest from Herefordshire 2 (10).

Figure 2.6 shows the relationship between population seed diameter and population 100 seed weight. This illustrates that the majority of seeds were between 0.0004 and 0.0005 g in weight and had a diameter of between 1.10 and 1.25 mm. The Caithness population was significantly larger in seed size. It is interesting to note that a linear relationship can describe the relationship between seed diameter and seed weight and that the two dimensions do not scale cubically as might be expected if the relationship between seed size and seed density remains constant.

Figure 2.7 shows that the predominant seed colour tended to be brown, but that the seed produced by the populations from Lanarkshire (16) and Perthshire (23) were significantly lighter.

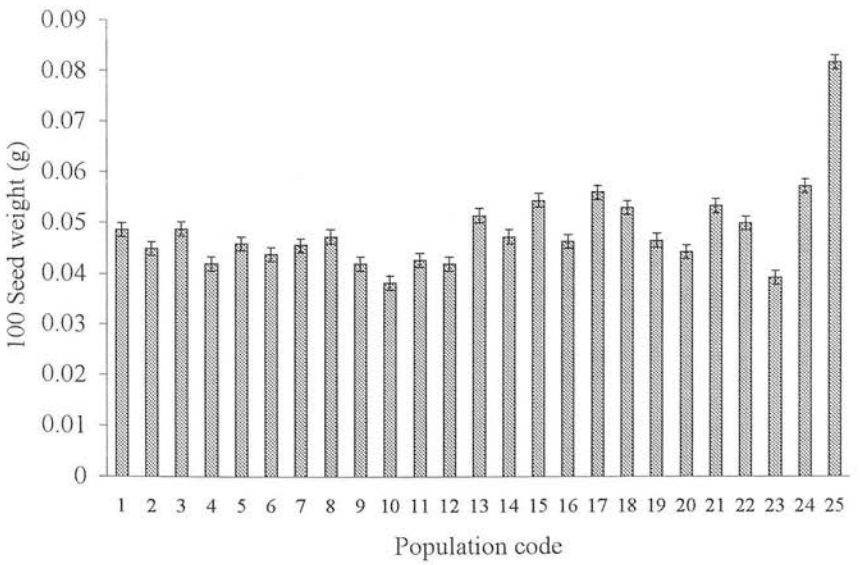


Figure 2.5: 100 seed weight for populations of *Stellaria media* produced in a common environment, August 1996.
n = 6; mean 100 seed weight ± 95% confidence interval.
Population codes as Table 2.2. $F_{(24, 125)} = 159.96$. $P < 0.0001$.

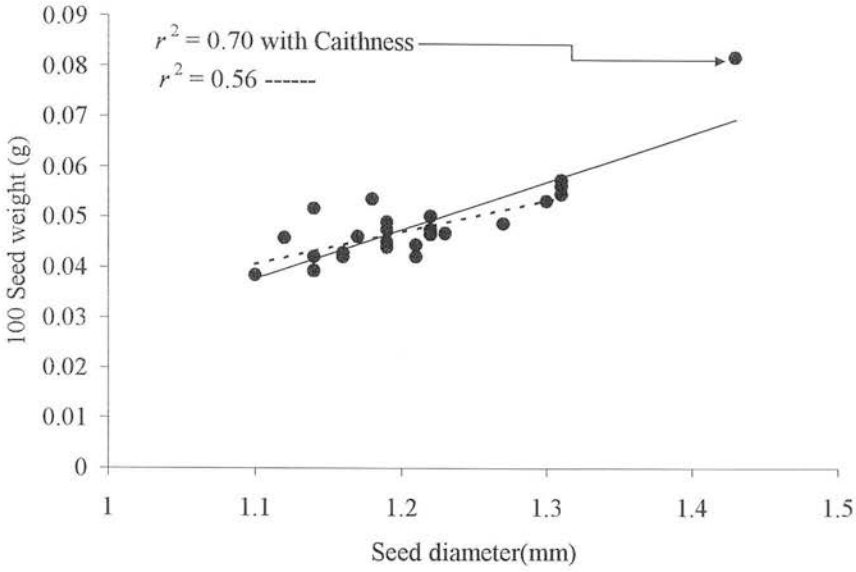


Figure 2.6: Mean seed diameter versus 100 seed weight for populations of *Stellaria media* produced in a common environment, August 1996.

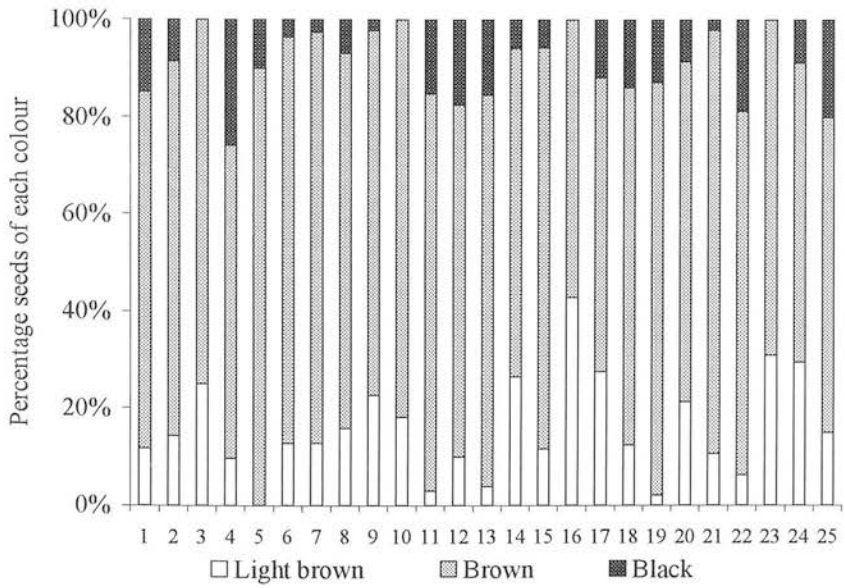


Figure 2.7: Seed colour on an arbitrary scale for populations of *Stellaria media* produced in a common environment, August 1996.
n >= 10; seed colour 1 to 3 (light brown to black).
Population codes as Table 2.2.
$\chi^2_{(48)} = 122.28$. $P < 0.0001$.

2.4.4. Screening for differences in seed germination characteristics

2.4.4.1. Analysis by fitting Gompertz curves to cumulated seed germination

Table 2.4 and Table 2.5 show the population parameters derived from fitting the Gompertz curve to the cumulated counts of germinated seeds. Table 2.3 gives a summary of the analysis of variance for difference between populations and treatments. Figure 2.9 and Figure 2.10 illustrate the fit of the Gompertz curve to the data and illustrate different germination time courses for two contrasting populations in each of the two temperature regimes.

The test temperature had significant effects on all 4 parameters for the fitted Gompertz curve and the value of t_{50}. The main effect of temperature on germination time courses is illustrated in Figure 2.8. Germination was less synchronous (except for the Nottinghamshire population (12)), slower and a smaller proportion of seeds germinated (except for Cambridgeshire 1 population (5)) in the colder regime.

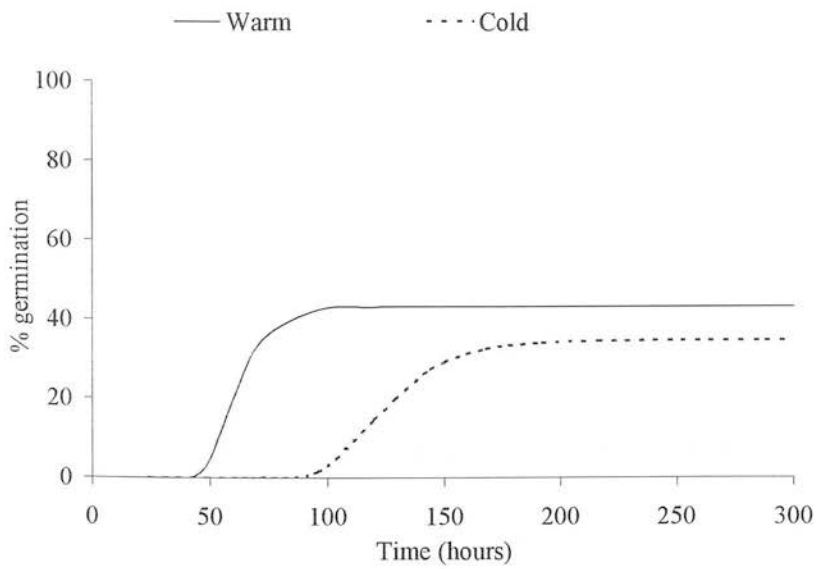


Figure 2.8: Fitted Gompertz curve comparing mean germination time courses for *Stellaria media* according to temperature.

In both temperature regimes, there were significant differences between populations for the parameters β, μ, γ and α and for the calculated value for t_{50}. With the exception of parameter β, there were no significant interactions between population and temperature. In the warm

temperature regime, germination was most synchronous for Leicestershire (7) and Stirlingshire (22) populations and least for Caithness (25) and Nottinghamshire (12). In the colder temperature regime, germination was most synchronous for Hampshire 1 (1) population and least for the population from Cheshire (13). This synchronicity of germination was not significantly correlated with the proportion of seeds that germinated in each population in each of the two temperature regimes (r^2 [20/15 °C; 74 df] = 0.009; r^2 [10/5 °C; 74 df] = 0.003).

In the warm temperature regime germination occurred fastest (as measured by parameter μ) in the Lothian 3 (19) population and this population together with the Hampshire 1 (1) and Stirlingshire (22) populations were the fastest in the colder regime. This contrasted with the slow speed of the Perthshire (23) and Cambridgeshire 1(5) populations in both temperature regime and the slow speed of the Caithness (25) population in the warmer regime. This speed of germination was not significantly correlated with the proportion of seeds that germinated for either of the two temperature regimes (r^2 [20/15 °C; 74 df] = 0.00006; r^2 [10/5 °C; 74 df] = 0.0019).

Factor	d.f.	β hr^{-1}		μ hr		$\gamma + \alpha$	
Population	24	3.27	***	2.18	**	30.22	***
Temperature	1	223.72	***	1032.58	***	60.80	***
Population x temperature	24	1.84	*	0.98	-	1.10	-
Residual	98						

(2 missing values)

Table 2.3: Summary of analysis of variance for the differences between populations for fitted Gompertz parameters (equation 2.1) according to test temperature for germinated *Stellaria media* seeds.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$

In terms of the proportion of seed that germinated, similar patterns occurred in both temperature regimes with the most seeds germinated from the Perthshire population (23) and the least from Cambridgeshire 1 (5), Kent (3) and Cheshire (13) populations.

Code	County	20/15 °C β hr^{-1}	μ hr	γ	α	t_{50} hr
1	Hampshire 1	0.1267	58.31	29.91	-0.073	61.25
2	Hampshire 2	0.1200	51.70	41.61	-1.183	55.13
3	Kent	0.1300	52.95	34.03	-0.943	56.10
4	Hertfordshire	0.1000	58.82	25.26	-0.463	63.03
5	Cambridgeshire 1	0.0900	64.74	8.07	-0.113	69.30
6	Cambridgeshire 2	0.1250	55.83	66.52	-0.955	58.93
7	Leicestershire	0.1333	56.82	34.12	-0.420	59.76
8	Norfolk	0.0967	54.58	47.03	-0.873	58.69
9	Herefordshire 1	0.1133	54.92	51.34	-0.870	58.42
10	Herefordshire 2	0.0700	61.20	33.51	-0.377	66.71
11	Warwickshire	0.1200	57.42	37.36	-0.700	60.76
12	Nottinghamshire	0.0667	60.39	53.31	-0.630	64.60
13	Cheshire	0.1067	54.84	24.11	-0.467	58.75
14	North Yorkshire	0.0967	57.90	27.57	-0.363	62.09
15	Borders	0.0767	52.76	58.21	-1.450	58.09
16	Lanarkshire	0.0833	63.41	50.77	-0.227	68.02
17	Lothian 1	0.0800	53.60	57.96	-1.507	58.72
18	Lothian 2	0.0867	54.54	70.46	-1.577	59.20
19	Lothian 3	0.1067	48.88	48.36	-0.210	52.54
20	Fife	0.1067	55.51	52.55	-0.893	59.37
21	Angus	0.1100	57.08	54.03	-0.750	60.67
22	Stirlingshire	0.1333	60.53	39.28	2.597	62.59
23	Perthshire	0.0867	66.58	81.00	1.437	70.90
24	Outer Isles	0.1167	54.87	29.53	-0.757	58.51
25	Caithness	0.0667	65.45	30.33	-0.163	71.07
	Mean	0.1036	57.33	43.74	-0.4884	61.33

Table 2.4: Mean parameters from fitting the Gompertz curve to cumulated counts of *Stellaria media* seeds germinating at 20/15 °C.
Population codes as Table 2.2; n = 3.

Code	County	10/5 °C β hr^{-1}	μ hr	γ	α	t_{50} hr
1	Hampshire 1	0.0867	97.15	17.70	-0.020	101.41
2	Hampshire 2	0.0700	111.06	29.29	-0.067	116.65
3	Kent	0.0467	126.30	17.48	0.017	134.88
4	Hertfordshire	0.0733	117.08	24.19	0.083	122.15
5	Cambridgeshire 1	0.0433	137.43	11.29	-0.027	146.06
6	Cambridgeshire 2	0.0500	121.74	51.08	-0.203	129.72
7	Leicestershire	0.0533	126.81	31.29	-0.083	134.03
8	Norfolk	0.0600	111.14	30.27	0.087	118.42
9	Herefordshire 1	0.0767	104.01	45.48	0.127	108.99
10	Herefordshire 2	0.0467	123.97	28.14	-0.003	132.86
11	Warwickshire	0.0500	116.16	21.18	-0.113	123.85
12	Nottinghamshire	0.0767	117.45	35.91	-0.010	123.31
13	Cheshire	0.0300	121.75	14.35	-0.167	134.65
14	North Yorkshire	0.0433	121.32	22.49	-0.140	130.67
15	Borders	0.0467	111.88	53.59	0.110	119.97
16	Lanarkshire	0.0400	128.31	36.25	0.413	137.07
17	Lothian 1	0.0533	105.86	50.00	0.023	113.00
18	Lothian 2	0.0567	117.56	49.65	0.047	124.16
19	Lothian 3	0.0533	98.56	44.66	-0.140	105.89
20	Fife	0.0600	111.62	36.04	0.223	117.72
21	Angus	0.0600	113.34	44.45	-0.003	119.45
22	Stirlingshire	0.0733	99.73	28.87	0.137	104.72
23	Perthshire	0.0333	137.14	79.62	0.247	148.24
24	Outer Isles	0.0567	114.27	23.54	0.187	120.67
25	Caithness	0.0367	115.92	22.64	0.043	126.49
	Mean	0.0538	117.10	34.66	0.033	124.73

Table 2.5: Mean parameters from fitting the Gompertz curve to cumulated counts of *Stellaria media* seeds germinating at 10/5 °C.
Population codes as Table 2.2; n = 3.

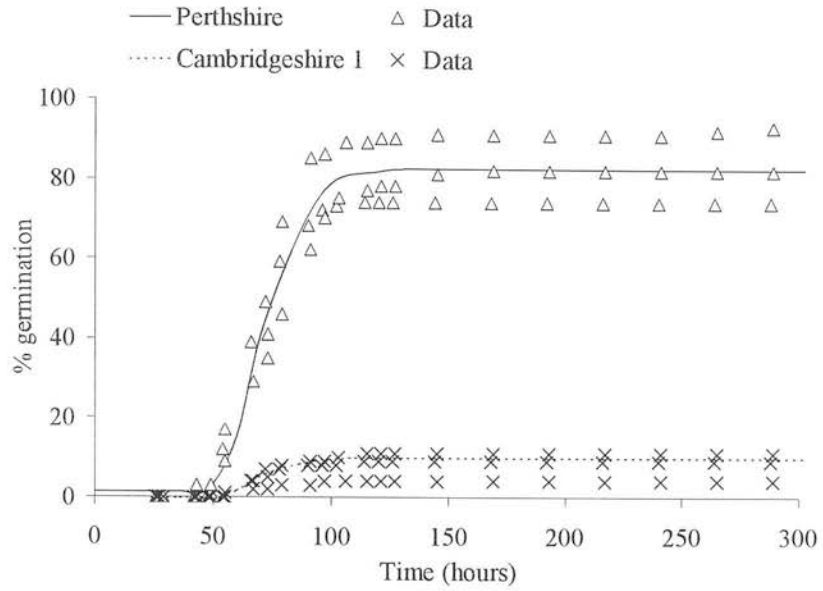


Figure 2.9: Fitted Gompertz curve compared with actual germination time course for two contrasting populations of *Stellaria media* at 20/15 °C.
n = 3.

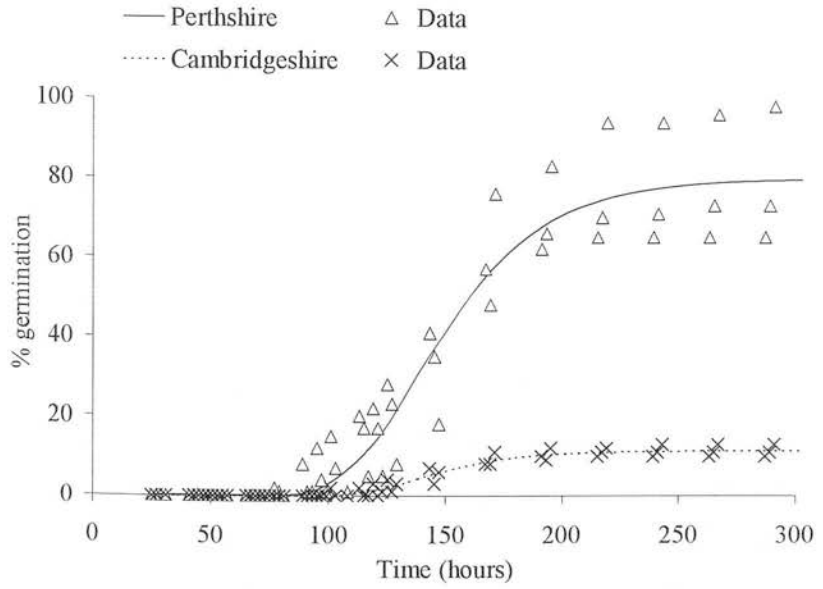


Figure 2.10: Fitted Gompertz curve compared with actual germination time course for two contrasting populations of *Stellaria media* at 10/5 °C.
n = 3.

2.4.4.2. Analysis by maximum likelihood methods

Table 2.6 and Table 2.7 show the population parameters estimated by the maximum likelihood approach. It was found that transforming time improved the model fit as assessed by the Wilks Likelihood Ratio (Hunter *et al.*, 1984). The best fit resulted from a log–lag transformation, that is log(*time* – *lag*), where the lag is also estimated by maximum likelihood. Table 2.8 gives of the analysis of variance for differences between populations and treatments. Figure 2.12 and Figure 2.13 compare the maximum likelihood predicted germination time course to the data and illustrate the different germination time course of two contrasting populations in each of the two temperature regimes.

In common with the Gompertz results, test temperature had significant effects on the speed of germination and proportion of seeds that germinated. The lag time and calculated value of t_{50} were also significantly different between temperatures, but the variance parameter was not. Germination was slower and a smaller proportion of seeds germinated (except for the Cambridgeshire 1 (5), Lanarkshire (16) and Perthshire (23) populations) in the colder regime.

Results for differences between populations were also similar to the Gompertz analysis. In both temperature regimes there were significant differences between populations for the estimated parameters m, p and *lag* and for the calculated value for t_{50}. There were no significant differences between populations for the estimated value for v.

The speed of germination as measured by m and t_{50} showed that the Kent (3) population germinated fastest in the warm temperature regime. The Lothian 3 (19) population was also fast to germinate as measured by t_{50} (but not m) in the warm regime. In the colder regime the Hampshire 1 (1) population was fastest to germinate as measured by both m and t_{50}. This contrasted with it being amongst the slowest populations to germinate in the warmer regime. The Lothian 3 (19) and Stirlingshire (22) populations were also relatively fast to germinate in the colder regime (as measured by t_{50}). According to both m and t_{50}, the Cambridgeshire 1 (5) and Lanarkshire populations were notably slow to germinate in the cold, and high mean times to germination (m) were also recorded for the Cheshire (13) and Perthshire (23) populations.

Code	County	20/15 °C m log(hr)	v log(hr)^2	p	lag (hr)	t_{50} (hr)
1	Hampshire 1	3.49	2.29	0.56	42	79
2	Hampshire 2	2.73	0.23	0.59	37	56
3	Kent	2.34	0.61	0.66	45	56
4	Hertfordshire	2.88	1.81	0.66	49	74
5	Cambridgeshire 1	2.82	0.89	0.89	55	72
6	Cambridgeshire 2	2.82	0.43	0.32	43	60
7	Leicestershire	2.67	0.51	0.65	45	60
8	Norfolk	3.06	0.53	0.50	37	62
9	Herefordshire 1	2.95	0.37	0.48	38	58
10	Herefordshire 2	3.23	0.63	0.65	40	68
11	Warwickshire	2.64	0.73	0.62	47	62
12	Nottinghamshire	3.37	1.24	0.27	47	82
13	Cheshire	2.74	0.63	0.75	43	59
14	North Yorkshire	3.04	0.48	0.72	39	63
15	Borders	3.45	0.29	0.42	27	62
16	Lanarkshire	2.97	0.72	0.46	47	68
17	Lothian 1	3.24	0.58	0.41	33	61
18	Lothian 2	3.36	0.54	0.23	33	63
19	Lothian 3	3.23	0.24	0.51	27	55
20	Fife	2.88	0.82	0.45	43	62
21	Angus	2.67	0.63	0.45	45	61
22	Stirlingshire	2.73	0.89	0.53	46	64
23	Perthshire	3.41	0.34	0.16	38	71
24	Outer Isles	2.63	0.68	0.70	45	59
25	Caithness	3.12	0.67	0.64	46	69
	Mean	2.98	0.71	0.53	41	64

Table 2.6: Estimated parameters from the maximum likelihood model for *Stellaria media* seeds germinating at 20/15 °C.
m is the mean time to germination.
v is the variance of the time to germination.
p is the proportion of the seeds failing to germinate.
Population codes as Table 2.2; n = 3.

Code	County	10/5 °C *m* log(hr)	*v* log(hr)^2	*p*	*lag* (hr)	*t*50 (hr)
1	Hampshire 1	3.10	0.80	0.80	81	105
2	Hampshire 2	3.44	0.69	0.68	87	121
3	Kent	3.60	0.74	0.80	90	129
4	Hertfordshire	3.42	0.66	0.74	91	124
5	Cambridgeshire 1	4.38	2.27	0.78	119	230
6	Cambridgeshire 2	3.69	0.61	0.44	91	134
7	Leicestershire	3.74	1.43	0.62	101	150
8	Norfolk	4.01	1.24	0.58	79	150
9	Herefordshire 1	3.39	0.67	0.52	79	110
10	Herefordshire 2	3.82	0.64	0.69	87	136
11	Warwickshire	3.63	1.14	0.76	91	130
12	Nottinghamshire	3.79	0.45	0.62	81	128
13	Cheshire	4.58	2.27	0.76	85	209
14	North Yorkshire	4.15	1.88	0.68	91	167
15	Borders	3.95	0.52	0.41	69	125
16	Lanarkshire	4.81	1.20	0.34	71	237
17	Lothian 1	3.87	0.40	0.48	67	118
18	Lothian 2	3.71	0.60	0.46	85	129
19	Lothian 3	3.61	0.60	0.53	69	108
20	Fife	3.65	0.50	0.62	79	118
21	Angus	3.60	0.40	0.54	83	121
22	Stirlingshire	3.44	0.54	0.70	71	106
23	Perthshire	4.45	0.72	0.06	75	165
24	Outer Isles	3.78	0.88	0.72	79	136
25	Caithness	4.07	0.99	0.73	75	136
	Mean	3.83	0.91	0.60	83	141

Table 2.7: Estimated parameters from the maximum likelihood model for *Stellaria media* seeds germinating at 10/5 °C.
Population codes as Table 2.2; n = 3.

Factor	d.f.	*m*		*v*		*p*		*lag*	
Population	24	2.20	**	1.24	-	14.36	***	3.29	***
Temperature	1	142.81	***	2.61	-	17.49	***	559.20	***
Population x temperature	24	1.86	*	1.33	-	1.69	*	1.12	-
Residual	95								

(5 missing values)

Table 2.8: Summary of analysis of variance for the differences between populations for maximum likelihood parameter estimates according to test temperature for germinated *Stellaria media* seeds.
F ratios with significance as *** P < 0.001; ** P < 0.010 and * P < 0.050.

Regarding the proportion of seeds that germinated, the highest values as estimated by (1- p) were again for the Perthshire (23) population and the lowest were for the Cambridgeshire 1 (5) population in both temperature regimes. Figure 2.11 shows the straight line relationship between estimates for final percentage germination from the maximum likelihood model and from fitted Gompertz curves. The gradient is not significantly different from 1, indicating good agremment between estimates.

Unlike the analysis of fitted Gompertz parameters, there were significant interactions between population and temperature for m, p and $t50$. This suggested that the populations differed in their germination response to the two different temperatures.

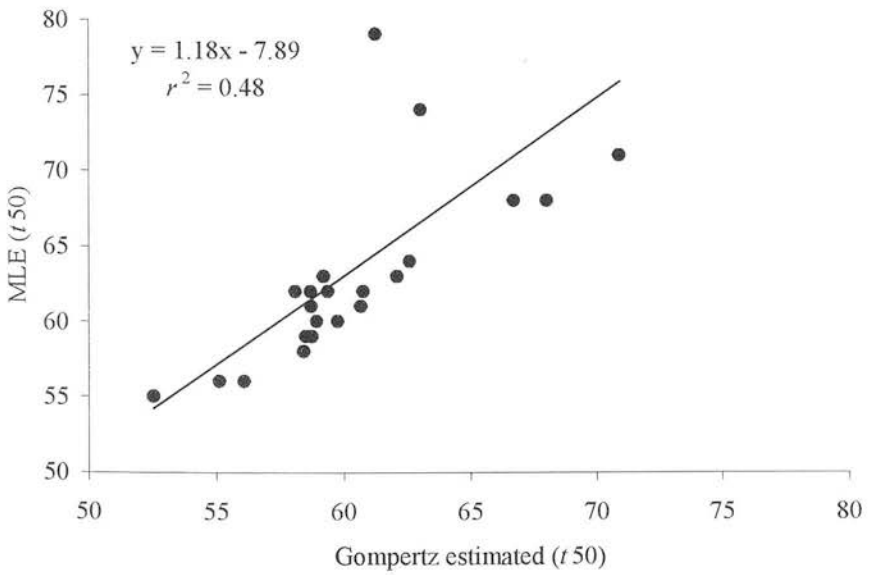


Figure 2.11: Relationship between estimates for final percentage germination from maximum likelihood model and from fitted Gompertz curves.

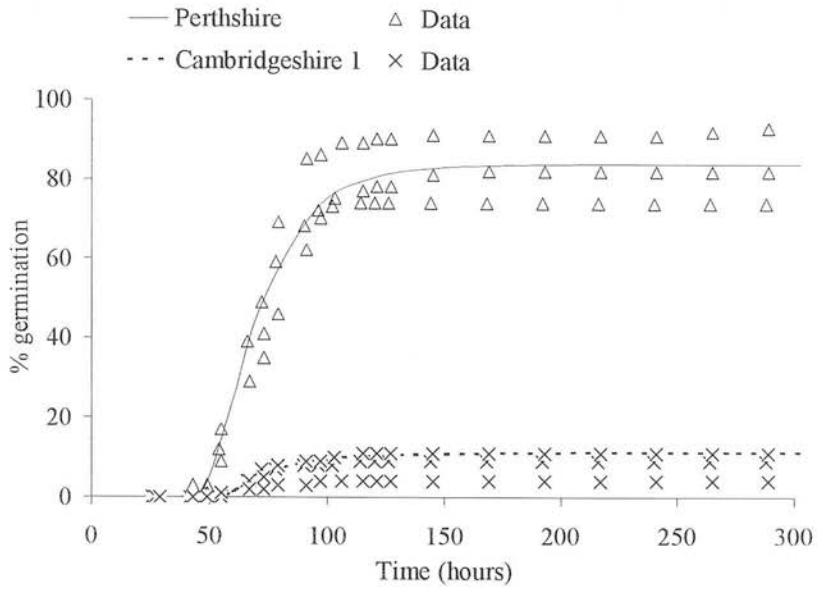


Figure 2.12: Maximum likelihood expected germination compared with actual time course for two contrasting populations of *Stellaria media* at 20/15 °C.
n = 3.

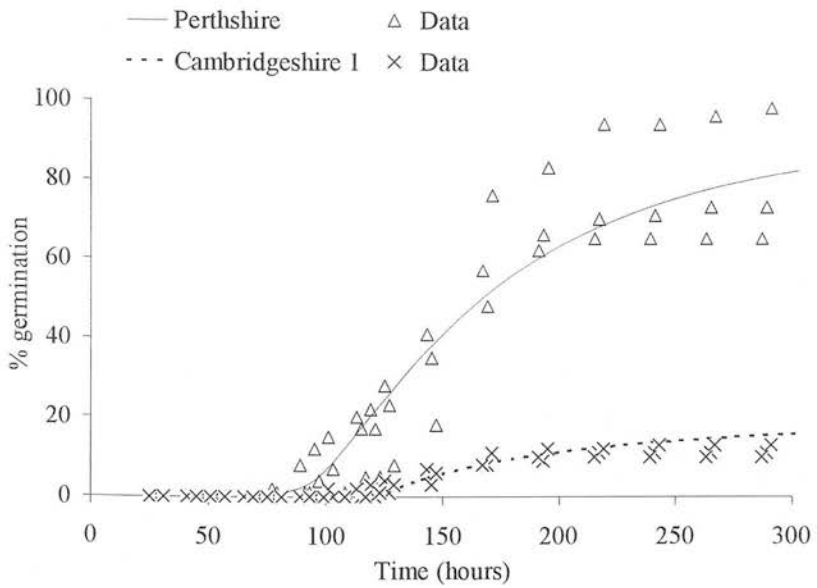


Figure 2.13: Maximum likelihood expected germination compared with actual time course for two contrasting populations of *Stellaria media* at 10/5 °C.
n = 3.

2.4.5. Screening for differences in seedling growth characteristics

Table 2.9 shows the mean population statistics from time of sowing (germination) to emergence, two leaf and four leaf stages in controlled conditions. Differences between populations in time from sowing to emergence and to two leaves were not significant. Overall seedling emergence occurred between five and seven days after sowing and the first two leaves were recorded after 11 to 13 days. Assuming a base temperature of 2 °C (see Chapter 6), this corresponded to a thermal time requirement of 80 to 110 degree days for cotyledon emergence and 175 to 205 degree days for emergence of first two leaves. There was a significant difference between populations for time from sowing to emergence of four true leaves. This varied from between 14 and 19 days (220 to 300 degree days). The Hampshire 2 population (2) was notably slow to produce four leaves in contrast to the Warwickshire (11) and Cheshire (13) populations.

Figure 2.14 and Figure 2.15 show the mean seedling height and width statistics for the populations. There were significant differences between populations for each of these statistics at both cotyledon emergence and emergence of the first two leaves. The Perthshire population (23) was the shortest at two leaves and the Caithness population (25) was the tallest.

Figure 2.16 shows differences between populations in dry weight recorded at emergence, two leaves and at four leaves. Although significant differences were recorded between populations for seedling dry weights at each instance, there were few consistent results. The Caithness (25) and Perthshire (23) populations were the only exceptions, consistently producing among the heaviest and lightest seedling dry weights respectively. The Lothian 2 population (18) produced the heaviest seedlings at emergence, but this was not reflected in later measurements. Missing values for the Cambridgeshire 1 population (5) reflected low levels of germination and the limited number of seeds available to grow on. Consequently, seedlings were not harvested at emergence or at two leaves. There was marked variation within populations for seedling dry weight at four leaves.

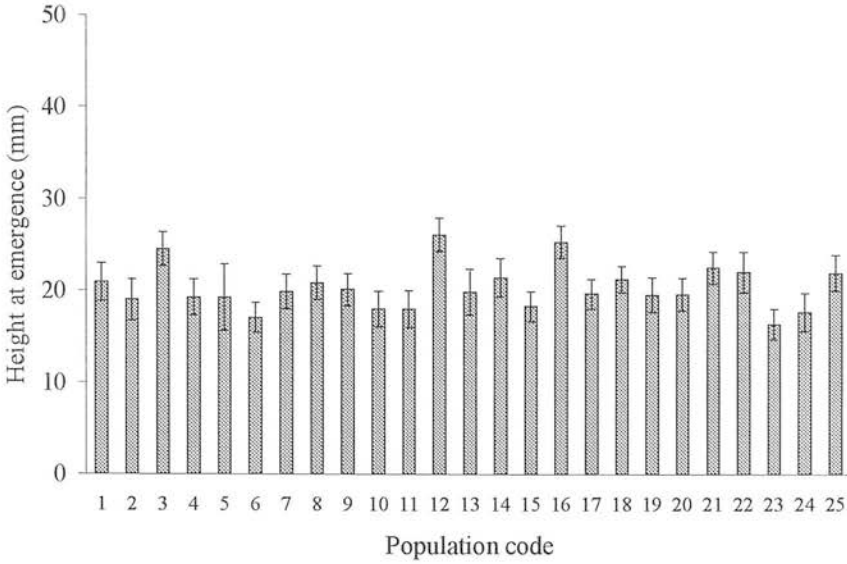
Figure 2.17 shows differences between populations in seedling relative growth rate for height from two to four leaves and for dry weight from sowing to two and to four leaves. Mean relative growth rates for seedling height from two to four leaves was highest for the Warwickshire population (11) and lowest for the Borders population (15). Again, although significant differences were recorded between populations for both seedling relative growth

rate and for dry weight, there were few consistent results. The relative growth rate for dry weight of seedlings from the Hampshire 2 (2) and North Yorkshire (14) populations tended to be slow. Again there was marked variation within populations for measurements of relative growth rate for seedling dry weight.

Code	County	t_1 (days)	t_2 (days)	t_4 (days)
1	Hampshire 1	6.78	13.39	17.50
2	Hampshire 2	7.18	13.79	18.71
3	Kent	7.21	13.25	16.87
4	Hertfordshire	5.10	11.67	15.00
5	Cambridgeshire 1	4.67	11.78	14.00
6	Cambridgeshire 2	5.48	12.55	15.83
7	Leicestershire	5.57	11.96	15.00
8	Norfolk	4.88	11.21	15.50
9	Herefordshire 1	5.90	12.20	14.25
10	Herefordshire 2	4.45	11.50	14.33
11	Warwickshire	6.14	11.71	13.67
12	Nottinghamshire	5.80	12.48	16.00
13	Cheshire	6.70	11.42	13.84
14	North Yorkshire	6.26	12.32	16.37
15	Borders	5.67	11.96	14.74
16	Lanarkshire	5.71	12.21	15.27
17	Lothian 1	5.84	11.87	14.31
18	Lothian 2	6.00	12.38	16.63
19	Lothian 3	6.18	12.30	15.23
20	Fife	6.87	13.70	16.71
21	Angus	5.97	12.55	15.71
22	Stirlingshire	6.92	12.43	16.00
23	Perthshire	6.21	12.58	14.14
24	Outer Isles	6.93	12.29	14.82
25	Caithness	5.47	11.25	15.11
	Mean	6.00	12.27	15.42
	F	1.00	1.00	3.70
	df	[24, 789]	[24, 530]	[24, 253]
	P	-	-	***

Table 2.9: Mean population seedling statistics for time from sowing (germination) to emergence (t_1), 2 leaves (t_2) and 4 leaves (t_4) for 25 UK populations of *Stellaria media*.
n = >10. *** P < 0.001; ** P < 0.010 and * P < 0.050.

h_1: $F_{(24, 680)} = 6.0$. $P < 0.0001$



h_2: $F_{(24, 429)} = 10.0$. $P < 0.0001$

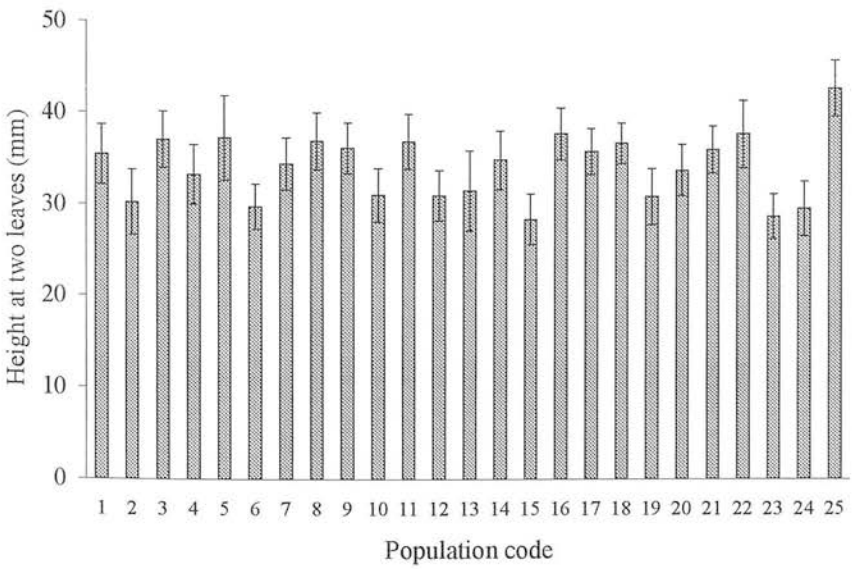
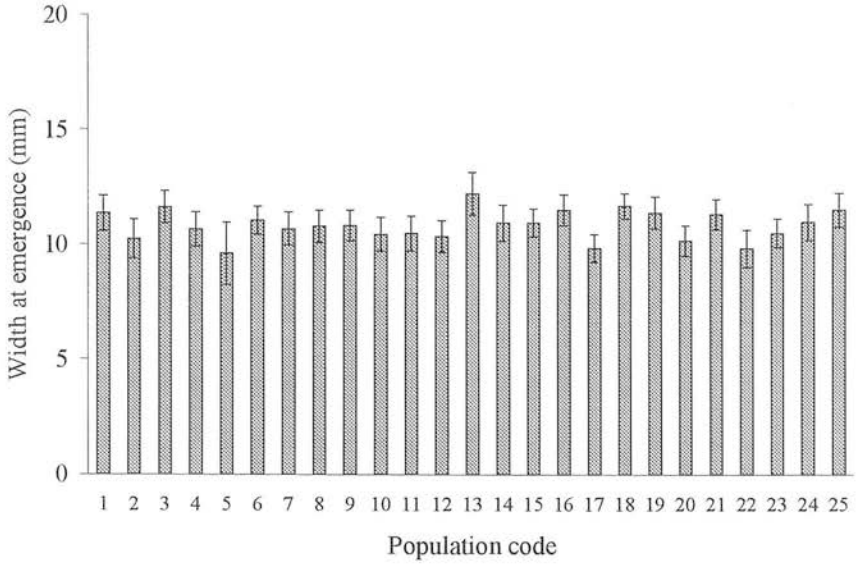


Figure 2.14: Seedling height at emergence (h_1) and at two leaves (h_2) for *Stellaria media* populations.
n >= 10; mean height ± 95% confidence interval.
Population codes as Table 2.2.

w_1: $F_{(24, 678)} = 3.4$. $P < 0.0001$



w_2: $F_{(24, 429)} = 4.8$. $P < 0.0001$

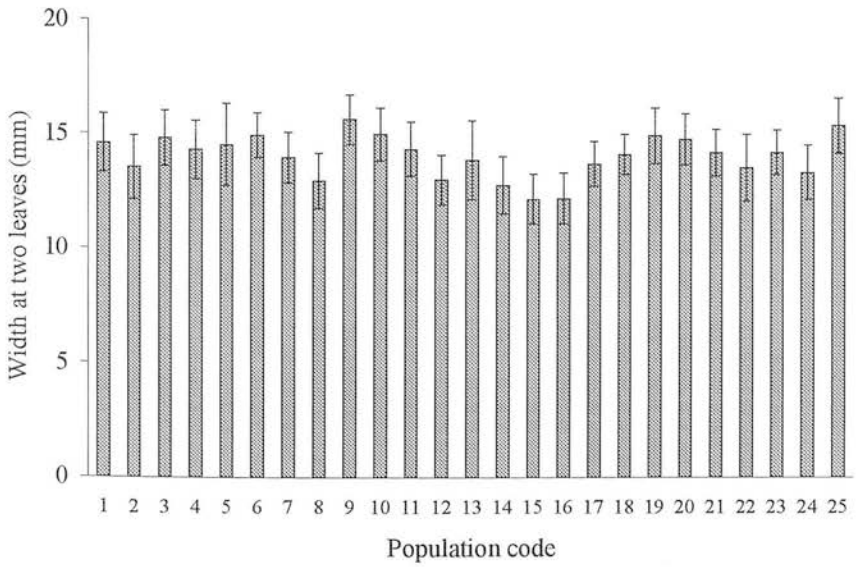
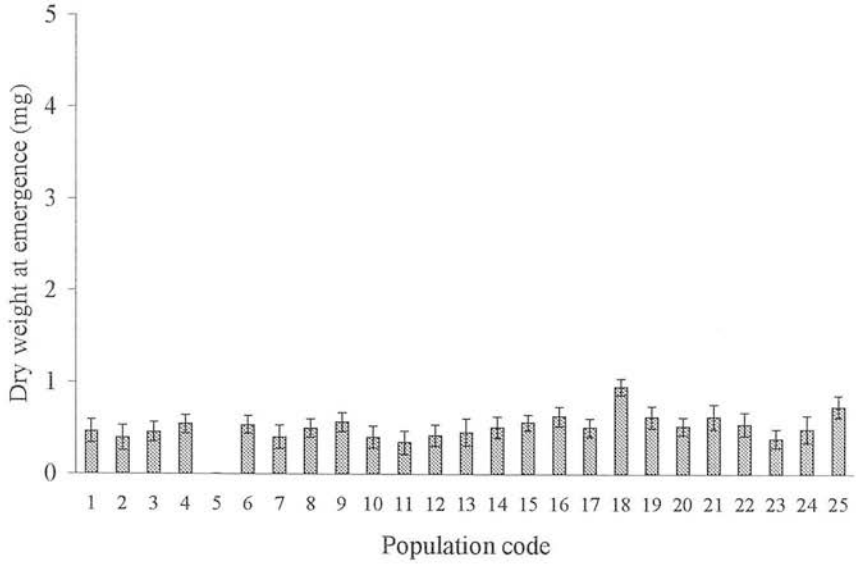
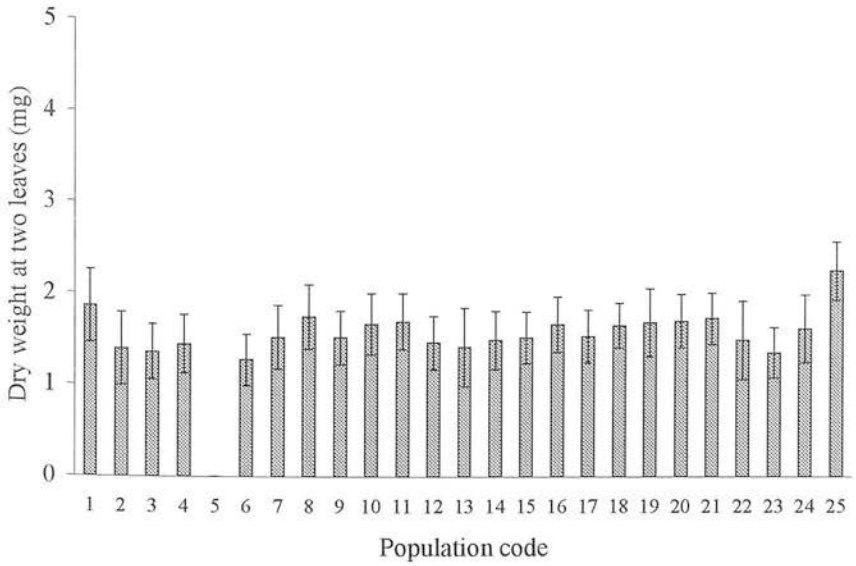


Figure 2.15: Seedling width across cotyledons at emergence (w_1) and across 2 leaves at two leaves emerged (w_2) for *Stellaria media* populations.
n >= 10; mean width ± 95% confidence interval.
Population codes as Table 2.2.

d_1: $F_{(23, 192)} = 12.05$. $P < 0.0001$



d_2: $F_{(23, 243)} = 5.87$. $P < 0.0001$



d_4: $F_{(24, 254)} = 4.6$. $P < 0.0001$

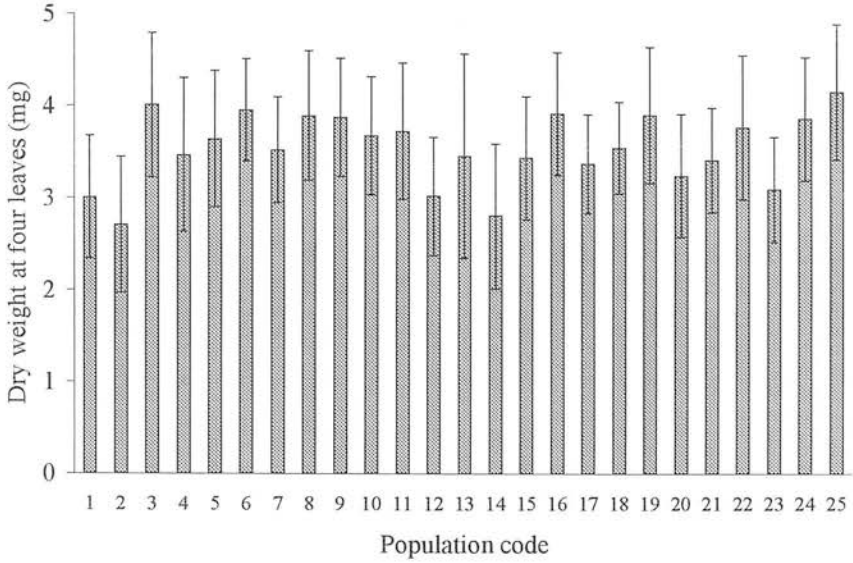
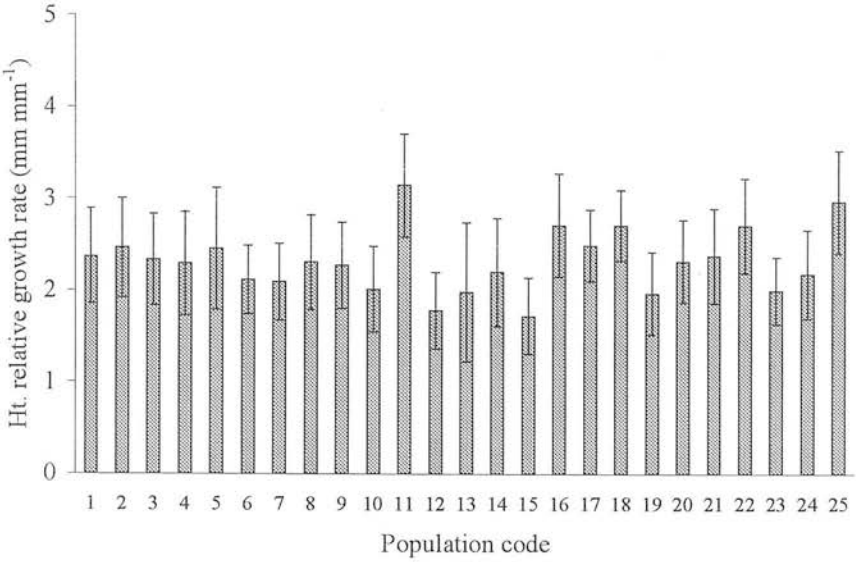
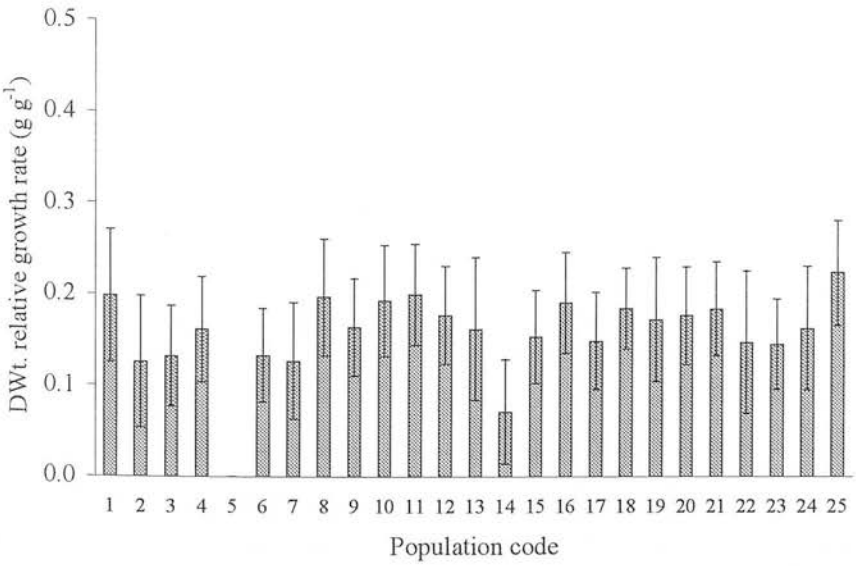


Figure 2.16: Seedling dry weight at emergence (d_1), at 2 leaves (d_2) and at 4 leaves (d_4) for *Stellaria media* populations.
n >= 5; mean width ± 95% confidence interval:
Population codes as Table 2.2.

r_h: $F_{(24, 357)} = 5.30$. $P < 0.0001$



r_{d2}: $F_{(23, 242)} = 4.22$. $P < 0.0001$



r_{d4}: $F_{(24, 245)} = 5.94$. $P < 0.0001$

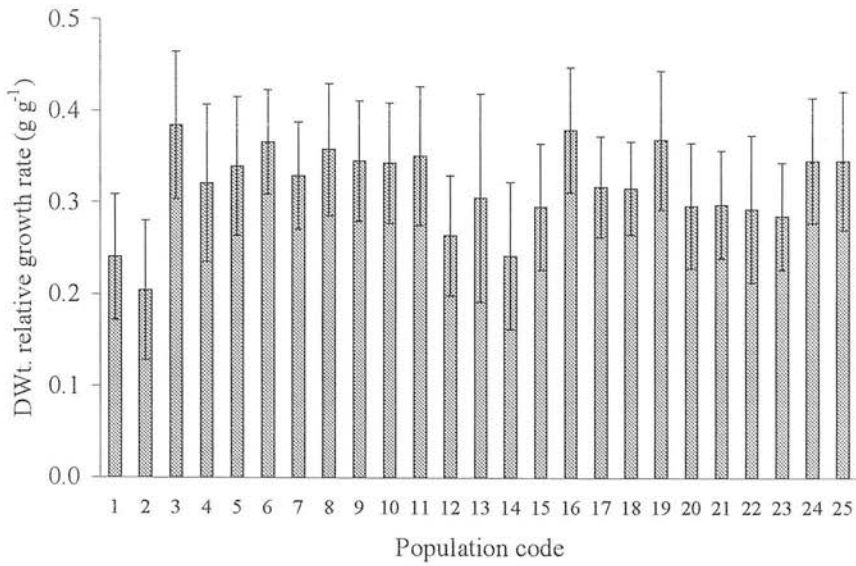


Figure 2.17: Seedling relative growth rates calculated for height gain from emergence to 2 leaves (r_h) and for dry weight gain from emergence to 2 leaves (r_{d2}) and to 4 leaves (r_{d4}) for *Stellaria media* populations.
n >= 5; mean width ± 95% confidence interval.
r_{d2} and r_{d4} calculated assuming d_1 = mean seed weight for each population.
Population codes as Table 2.2.

2.4.6. Prediction of dry weight and relative growth rates from other seedling characteristics

Partial least squares regression of the seedling growth statistics was applied with seedling dry weights and relative growth rates for dry weight as the dependent variables. Representative results for seedling dry weight at four leaves and seedling growth rate to four leaves are shown in Table 2.10. Cross validation showed that only factor 1 was relevant for prediction. Factor 1 explained some 40 % of the total variation. The analysis showed that for seedling dry weight at four leaves and seedling growth rate to four leaves, time to two leaves was the variable most closely related to factor 1. For seedling dry weight at four leaves, height at two leaves was also associated with factor 1, whilst for seedling relative growth rate to four leaves, the timing of emergence and time to four leaves were associated. Seed diameter, seed colour and seedling width statistics were not important components of factor 1. Table 2.10 shows the difficulty in predicting seedling dry weight and relative growth rate from non-destructive measurements.

Dependent variable	d_4	r_{d4}
% total variance of dependent variable explained by factor 1	44.3	43.7
Independent variable	% total variance explained by factor 1	
Seed diameter (*sd*)	8.3	0.4
Seed colour	0.7	0.5
Height at emergence (h_1)	14.1	4.4
Height at two leaves (h_2)	42.3	16.4
Height relative growth rate (r_h)	25.5	6.7
Width across cotyledons at emergence (w_1)	17.5	15.5
Width across first two leaves at two leaves (w_2)	1.3	0.2
Time from sowing to emergence (t_1)	25.4	43.9
Time from sowing to two leaves (t_2)	57.2	73.2
Time from sowing to four leaves (t_4)	26.7	50.5

Table 2.10: Partial least squares regression analysis of variance for *Stellaria media* seedling characteristics using (a) seedling dry weight at four leaves (d_4) and (b) seedling relative growth rate to four leaves (r_{d4}) as dependent variables.

2.4.7. Selection of contrasting populations

Figure 2.18 shows the similarity matrix calculated from mean population statistics for seed size, seed colour, seed germination and seedling growth statistics (details in Table 2.1). The similarity matrix shows that on the basis of the data entered, the populations from Hampshire 2 (2) and Cambridgeshire (5) were the most dissimilar (69 %) and that the populations from the Borders (15) and Lothian 1 (17) and from Fife (20) and Angus (21) were the most similar (97 %). It is interesting to note that the two Hampshire populations (1 and 2) were 86 % similar; the two Cambridgeshire populations (4 and 5) were 87 % similar; the two Herefordshire populations were 93 % similar and the three Lothian populations (17, 18 and 19) were between 94 and 96 % similar.

P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	----																								
2	86	----																							
3	81	88	----																						
4	91	85	88	----																					
5	71	69	77	87	----																				
6	80	88	92	90	78	----																			
7	84	89	94	94	89	95	----																		
8	84	84	90	94	86	93	95	----																	
9	85	87	92	93	77	95	94	95	----																
10	83	83	87	95	87	92	94	95	93	----															
11	84	85	91	94	87	91	96	95	93	93	----														
12	88	91	86	95	81	92	94	94	93	93	91	----													
13	76	82	87	86	88	86	92	90	83	88	89	85	----												
14	83	91	88	89	87	87	95	90	87	89	88	91	92	----											
15	81	86	84	89	74	93	90	94	92	92	87	95	84	89	----										
16	70	70	83	82	85	82	86	91	83	86	83	83	84	85	84	----									
17	84	88	88	91	77	94	92	96	96	93	92	94	83	89	97	85	----								
18	86	88	89	89	74	94	90	95	93	90	90	93	84	88	95	86	96	----							
19	82	88	90	88	70	95	90	93	95	90	89	89	86	85	93	77	94	94	----						
20	90	95	93	92	77	94	93	93	96	91	92	95	84	90	93	83	95	95	93	----					
21	88	91	93	93	79	95	95	96	97	93	93	95	87	90	94	87	96	97	94	97	----				
22	89	90	90	91	76	89	91	90	92	86	91	91	82	87	86	79	90	90	90	94	94	----			
23	71	77	74	80	72	88	84	83	84	86	79	88	76	82	88	83	87	86	79	86	85	78	----		
24	85	89	95	92	82	93	95	95	94	92	94	92	91	91	93	86	93	91	93	95	95	92	81	----	
25	79	71	81	85	84	80	82	90	84	87	87	80	81	79	82	84	86	89	84	83	88	83	70	86	----

Figure 2.18: Similarity matrix for *Stellaria media* populations calculated from mean seed size and colour, seed germination and seedling growth statistics.
Statistics entered as Table 2.1.
Population codes as Table 2.2.

Furthest neighbour cluster analysis based on the similarity matrix in Figure 2.18 is shown in Figure 2.19. Cluster analysis identified the Caithness (25) and Perthshire (23) populations as representing markedly contrasting populations and suggested that the Cambridgeshire 1 (5), Cheshire (13), North Yorkshire (14) and Lanarkshire (16) populations were also atypical.

Ranking the populations for each statistic and summing the number of top five and bottom five rankings validated the selection of the Caithness (25) and Perthshire (23) populations as contrasting 'extreme' populations. The Perthshire population had the highest score and the Caithness population was fifth. The Leicestershire (7) and Angus (21) populations were equally low scoring and the Leicestershire population was selected as the most typical population on the basis that this maximised the geographic spread of the selected populations.

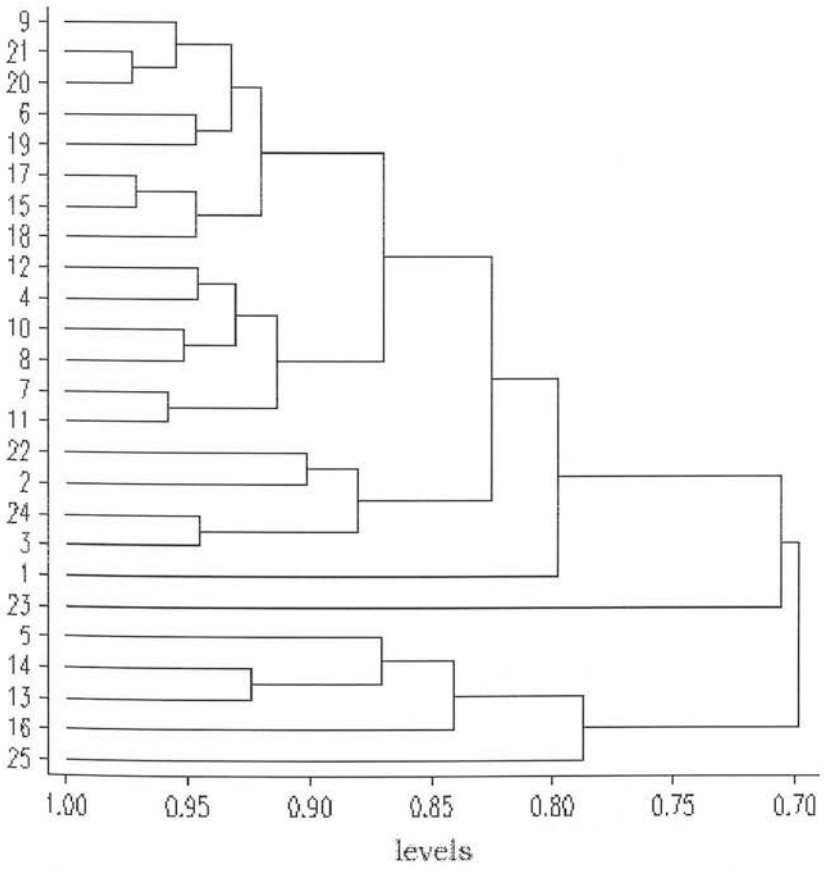


Figure 2.19: Furthest neighbour cluster analysis of *Stellaria media* populations according to seed germination and seedling growth statistics.
Population codes as Table 2.2.

2.5. Discussion

2.5.1. Seed collection and sampling strategy

Table 2.2 shows that seed was collected from a wide range of locations across the UK, albeit with a bias to areas of arable cropping and with a relatively high representation of sites in central Scotland. The bias to areas of arable cropping was not considered serious as this project was devised to assess the characteristics of *Stellaria media* in the context of better understanding the biology of this species as a weed of arable crops. Therefore although *S. media* occurs in other areas, differences in the characteristics of these populations that have evolved under different environmental conditions were considered less important for this study. The bias to central Scotland perhaps gives greater concern, although there is ample evidence in the data to document that differences between populations can occur on a wide range of scales and that geographically widespread populations are not necessarily as dissimilar as populations within a region that are exposed to very different conditions. This has also been demonstrated in other studies including Crawford & Jones (1977) and Warren *et al.* (1998). Moreover, as a large number of populations were included in this initial screening and it was hoped that this would also dilute any minor geographical bias.

2.5.2. Seed replication and initial observations

It was clear from initial observations of seedling emergence and flowering times, that populations differed, although the extent to which this reflected actual genetic differences could not be assessed. It was interesting to note that differences in seedling emergence did not appear to reflect later recorded differences in final percentage germination or differences in latitude. However the delayed flowering of the Perthshire (23) population appears to be a defining characteristic of that population.

It was also noted that generally there was a poor correlation in seedling emergence statistics between populations collected on the same date at nearby locations (e.g. Hampshire 1 (1) and 2 (2) and Herefordshire 1 (9) and 2 (10)), but that there was a generally close correlation between percentage emergence and time to emergence, with populations exhibiting high percentage emergence also emerging earlier than populations with low emergence.

2.5.3. Differences in seed physical characteristics

Previously quoted mean seed weights have varied from 0.35 mg (Grime, Hodgson & Hunt, 1988) to 0.67 ± 0.04 mg (Sobey, 1981). This range encompasses all of the mean seed weights recorded in the study, except for the population from Caithness that was clearly larger than those previously recorded. The Caithness population was also the population with the widest seed diameter and when this data was included the linear relationship between seed diameter and seed weight was more pronounced. It was observed that there was perhaps a tendency for the Scottish populations (except Perthshire) to produce larger, heavier seeds but a reciprocal transplant experiment would be needed to test whether this difference was simply the result of local adaptation to the Scottish common environment. It was observed that lighter colour seeds were associated with the populations from Lanarkshire (16) and Perthshire (23), both of which were also relatively late to flower in spring 1996.

2.5.4. Differences in seed germination characteristics

The effect of temperature on the proportion of seeds germinating and the synchronicity of germination can be considered to mirror the pattern of autumn and spring flushes of *S. media* emergence in the field (Roberts & Feast, 1973; Sobey, 1981). Using both methods of analysis, it is clear that whilst colder winter temperatures do not completely inhibit seedling emergence, fewer seedlings establish, more slowly and with little synchronicity in emergence. Cambridgeshire 1 (5) was the only population for which more seeds germinated in the colder temperature regime and Nottinghamshire (12) was the only population for which the synchronicity of germination was greater in the cold. The total Cambridgeshire 1 (5) germination was low and as such significantly greater cold germination was only relative and unlikely to result in greater weed problems in the field. The low Nottinghamshire (12) synchronicity in the warm temperature regime is likely explained by a tendency for a double peak in germination. This characteristic was restricted to the Nottinghamshire (12) population and could lead to greater weed problems in the field as a result of weed control measures targeting the initial population of seedlings germinated, allowing the later seedlings to escape. However over the timescale involved (< 14 days) this is unlikely to cause a problem.

Both methods of analysis showed that the Perthshire population (23) population had the highest and the Cambridgeshire 1 (5) the lowest final percentage germination in the two

temperature regimes. There did not appear to be any association between seed size or colour characteristics and seed germination statistics. It was reassuring to show the close correlation between final % germination predicted by the two methods (Figure 2.11) and the similarity in the fitted curves (e.g. Figure 2.9 compared with Figure 2.12). Therefore although it can be argued that the maximum likelihood model is a more statistically rigorous method of analysing seed germination curves (given that it makes fewer assumptions about the shape of the curve and allows the calculation of error terms for the fitted model), the greater ease and more ready interpretation of the Gompertz parameters (without the need for log (time-lag) calculations) meant that this model was preferred for later analyses. However, it was interesting to note that whereas interactions between population and temperature, with the exception of β, were not significant for the fitted Gompertz curve parameters, interactions for each of the parameters predicted by the maximum likelihood model were significant. It was not clear why this should be the case.

It was clear from analysis of the final percentage germination statistics that a large number of seeds were left ungerminated in many of the populations surveyed. It was initially unclear whether this reflected differences in seed viability or differential levels of seed dormancy between populations and therefore tetrazolium tests (methods described in Ellis, Hong & Roberts, 1985) were applied. The tetrazolium tests (results not presented) showed that the remaining seeds were > 95% viable and this underlined the need for further investigation of the factors controlling germination.

2.5.5. Differences in seedling growth characteristics

It was interesting to note that the Caithness population, in addition to producing the largest seeds, also produced the largest and heaviest seedlings and some of the highest growth rates. For other populations the relationship between mean seed size, weight and seedling growth statistics was less pronounced, although again there was perhaps a tendency for the Scottish populations to produce larger, heavier seedlings at emergence.

Grime *et al.* (1988) quote seedling growth rates ranging between 0.246 and 0.343 g g $^{-1}$ for the five weeks from emergence. These values are comparable to the values calculated for seedling growth from emergence to four-leaves. The values calculated for seedling growth from emergence to two leaves tend to be smaller than this and further investigation is

required to ascertain whether these growth rates are actually slower or whether this is a result of greater inaccuracy in the smaller measurements made at this growth stage.

2.5.6. Difficulties in predicting seedling dry weight and relative growth rates

The partial least squares regression analysis demonstrated the difficulty of predicting seedling dry weight and relative growth rate from other seed and seedling characteristics. Again this may relate to the inaccuracies in measurement of seed and seedling physical characteristics or it may reflect differences in seedling structure and chemical composition that might prevent general predictions.

2.5.7. Final selection of contrasting populations

The final selection of populations was unweighted, except by the information included. It was clear from the final analysis that there was no general geographical patterning in the data in as much that on the basis of the entered data, the two Hampshire populations were as dissimilar from each other as they were from the population from the Outer Isles and the two populations from Herefordshire were as dissimilar from each other as they were from populations in Lothian. It was reassuring that the two methods gave similar results identifying the Caithness (25) and Perthshire (23) populations as contrasting and the Leicestershire population as 'average'.

2.6. Conclusions

Given that seed was harvested from plant grown for a generation in a common environment, the significant variation between the different populations of *S. media* in germination and seedling growth can largely be attributed to genetic differences between populations. There was no geographical pattern that correlated with the differences observed. Instead it is likely that the differences in seed size, seed germination and seedling growth characteristics result from complex interactions between the maternal and test environments and the genetic structure of the populations. Different responses would be expected if the seeds had been produced in a different maternal environment or if the seeds had been tested under different conditions. The magnitude of differences in germination and seedling growth characteristics observed in this study suggest that future studies aimed at describing the ecology of *S. media* need to consider the extent of inter-population variability. It is also clear that the observed

variability between populations of *S. media* has important implications for commercial and agronomic work on developing methods and techniques of weed control.

Chapter 3. The selection of contrasting populations of *Galium aparine L.*

3.1. Summary

Seeds from 38 populations of *Galium aparine* were collected from arable field sites and grown for a generation in a common environment. Using second generation seed to limit maternal sources of variation, significant differences between populations in physical seed characteristics, germination patterns and seedling growth statistics were recorded. These characteristics were used in a multivariate cluster analysis to select three contrasting populations to use in later, more detailed studies.

This chapter also uses partial least square regression analysis to identify possible physical predictors of seedling dry weight and seedling relative growth rate.

3.2. Introduction

Chapter 2 showed that there were significant differences between populations of *Stellaria media* in physical seed characteristics, seed germination and seedling growth. This chapter will apply the same screening methodology to assess the scale of variation between populations of *Galium aparine*.

G. aparine is a widespread winter annual species that commonly occurs as a weed of arable crops. It occurs in hedgerow habitats and previous work has shown significant differences in the ecology of field and hedgerow populations. These include morphological differences (Froud Williams, 1985; Ferris, 1988; Bain & Attridge, 1988; Froud-Williams & Ferris-Kaan, 1991) and physiological differences including greater seed production and higher seed dormancy in field populations (Froud Williams, 1985; Ferris, 1988; Froud-Williams & Ferris-Kaan, 1991), vernalisation requirements for flowering in hedgerow populations (van der Weide, 1992). Mitchelson *et al.* (1995) have additionally identified patterns of genetic varition that may be linked to adaptations to agricultural land. Consequently it is important to distinguish between *G. aparine* populations from the two different habitats and therefore it should be noted that populations compared in this investigation were restricted to populations collected from arable field habitats.

Differences in *G. aparine* germination have previously been recorded for six arable populations in the UK (Ferris, 1988). The populations differed markedly in morphology and germination responses to temperature, light and nitrate availability. However these populations were of English origin and appeared to have been selected arbitrarily. More importantly the populations were compared without effort to limit the effect of differences in maternal environment.

It was therefore considered useful to conduct a widescale survey of field populations of *G. aparine* in which variation between populations was assessed on a common basis, using seeds from plants grown for a generation in a common environment. In addition to describing the extent of variation in physical seed characteristics, seed germination and seedling growth statistics, multivariate cluster analysis was used to identify three contrasting populations of *G. aparine*, which could then be used in further investigations.

3.3. Methods

3.3.1. Seed collection

Over the summer of 1995, seeds from field populations of *G. aparine* were collected at maturity from 22 arable sites in the UK (more than 10 m from field boundary). Seeds were collected from a large number of plants at each site and were allowed to dry at room temperature for approximately two weeks. The cleaned seed (in paper envelopes) were then transferred to an incubator maintained at 10 °C (± 2 °C), with a tray of silica gel placed within the incubator to reduce humidity. Each seed collection was labelled with site details including field name, farm name and National Ordnance Survey (OS) grid reference, together with harvest date and name of collector. Additional information on cropping history was recorded where available.

Seeds from an additional 16 field populations were harvested over the summer in 1994 from sites across the UK and Europe for use in a population genetic survey. This seed was donated by Martin Ford of SAC Aberdeen and on receipt this seed was transferred to the same incubator maintained at 10 °C. Some of this seed originally derived from collections of Alan Hill in Northern Ireland and Rommie van der Weide in the Netherlands.

3.3.2. Seed replication in a common environment

Seeds were multiplied in a common environment starting in October 1995 (with the exception of the Edinburgh (34) population that was sown out using the same methods in November 1995). Twenty seeds from each population were sown in three replicate pots. The pots were randomly arranged in three blocks in an unheated greenhouse and pots were watered regularly. Seedling emergence was recorded and the seedlings thinned to 5 per pot. In January 1996, the plants were transferred to a heated greenhouse (target temperature 10 °C) following exceptionally low temperatures in late December. As the plants grew, canes and twine were used for support and to restrict interaction between neighbouring pots. On first flowering, pots were covered in a layer of muslin, sealed at the bottom with an elastic band, to prevent cross fertilisation between populations. Mature seed was collected in August 1996, dried, cleaned and stored as described above.

3.3.3. Screening for differences in germination characteristics

Starting in March 1997, the germination characteristics of populations that produced seed were assessed in two controlled environment cabinets (FISONS), each maintained with a 14 hr day. The warm cabinet (W) was 20 °C during the day and 15 °C at night and the cold cabinet (C) was 10 °C during the day and 5 °C at night. The irradiance during the day varied between a photosynthetically active photon flux density (PPFD) of 160 to 205 μmol m^{-2} s^{-1} (as recorded using a quantum sensor and a Campbell CR10 datalogger). To counter the lack of replication of the controlled environments, replicates were started at approximately 10 day intervals. For each of the three replicates, 75 seeds were counted, weighed and were placed on 2 layers of filter paper (Whatman Grade 181) in a 9 cm Petri-dish. The papers were then moistened with 10 ml of distilled water and placed in a randomised block on one shelf in the cabinet. The seeds were checked after 24 hrs, then twice daily for the next 4 days, (coinciding with peak of germination) followed by further daily checks for another 16 days. The Petri-dishes were inspected in the same order on each occasion. The Petri-dishes were removed and recorded at room temperature under fluorescent light. Seeds that had germinated (as defined by radicle emergence > 1 mm) were counted and removed.

For statistical analysis of germination time courses, the Gompertz curves were fitted to the cumulated seed germination data for each Petri-dish from the warm cabinet by least squares (Genstat 5). The Gompertz curve was defined as in section 2.3.3.1. and was also used to estimate time to 50 % germination (t_{50}) for each data set. Analysis of variance (Genstat 5)

was used to identify differences in the fitted parameters of the Gompertz curve and estimated t_{50} between populations, excluding data sets where the Gompertz curve was poorly fitted ($r^2 < 0.9$).

3.3.4. Screening for differences in seedling growth characteristics

A subset of the first seeds to germinate in the warm cabinet were transferred to a growth room maintained with the same temperature and day length conditions (14 hr day; 20/15°C). PPFD varied between 75 to 90 μmol^{-1} m^{-2} s^{-1}. A minimum of 50 germinated seeds per population were transferred and sown individually into 30 mm diameter cells in 10 x 15 cell-trays filled with potting mixture (John Innes no. 2). Seed diameter was measured to the nearest 0.5 mm at sowing, using callipers. Seed colour was recorded on an arbitrary scale of 1 to 4 (light brown to black) and radicle length at sowing was recorded. Seedling height and width across the first whorl was measured at the 1 whorl stage. The time taken from sowing to emergence and to the 1 whorl stage was recorded for each individual. Established seedlings were harvested at the 1 whorl stage and the dry weights of these seedlings recorded. Seedling relative growth rates were calculated by subtracting the natural log of mean population seed weight from the natural log of each seedling dry weight and dividing by the difference in days to emergence and days to harvest at 1 whorl (methods follow Hunt, 1978a, b). Analysis of variance (Genstat 5) was used to identify differences between populations for each of the seed and seedling characteristics.

3.3.5. Prediction of dry weight and relative growth rates from other seedling characteristics

Partial least squares regression was used to investigate the potential to predict seedling dry weight and relative growth rates from other seed and seedling characteristics. The PLS method (Genstat 5, with program supplied by Dr. Mike Talbot) was introduced in section 2.3.5. and this was applied to data in a 10 x 8 matrix of 10 populations and eight independent variables. These were seed diameter, seed colour and seed radicle length; seedling height and width at 1 whorl; the number of leaflets in the first whorl and time from sowing to emergence and to 1 whorl. The 10 x 2 matrix of 2 dependent variables were seedling dry weight at 1 whorl and seedling relative growth rate to 1 whorl. The columns of both matrices were transformed to zero mean and unit variance before the analysis.

3.3.6. Selection of contrasting populations

In order to select contrasting populations for further study, a similarity matrix was constructed from the combined seed size, seed colour, seed germination and seedling growth statistics. The similarity matrix was then entered into a furthest neighbour cluster analysis (Genstat 5). The statistics entered are listed in Table 3.1. The statistics were unweighted. The aim was to identify three populations, two representing contrasting behaviour and characteristics and one representing a 'typical' *G. aparine* population. This was done by identifying the lowest level at which three clusters were represented and selecting populations within each cluster. The 'normal' population was selected from the largest cluster.

The results from cluster analysis were checked by ranking populations for each statistic and summing the number of top five and bottom five rankings to identify the two most 'extreme' populations and the one 'typical' population.

Seed	Seed germination	Seedling growth
• 100 seed weight (g)	• Percentage germination	• Time from sowing to emergence
• Seed diameter (mm)	• Gompertz parameter β	• Time from sowing to 1 whorl
• Seed colour	• Gompertz parameter μ	• Height at 1 whorl
• Seed radicle length	• Gompertz parameter α	• Width at 1 whorl
	• Gompertz parameter γ	• Number of leaflets in first whorl
	• Time to 25 % germination	• Dry weight at 1 whorl
	• Time to 50 % germination	• Relative growth rate from sowing to 1 whorl
	•Time to 75 % germination	

Table 3.1: List of data entered into cluster analysis for selection of contrasting populations.
Data were population means.
Data for seed germination were from the warm test environment.

3.4. Results

3.4.1. Sites

Table 3.2 shows the location and description of sites from which the 38 populations were collected. The sites ranged in latitude from 44° 0′ to 58° 30′ N. It should be noted that sites were biased to areas of arable cropping and that a relatively large number of sites in southern England were represented. It should also be noted that samples were largely taken from

experimental farms, especially in England. All the sites were in arable rotations and with the exception of those marked with an asterix (*) were more than 10 m from a field boundary. Where site details were given, elevation ranged from 0 to 280 metres above mean sea level and samples were collected from crops including winter wheat, winter barley, spring wheat, linseed, cabbages, broccoli, turnips and rotational set-aside.

3.4.2. Seed replication

Figure 2.1. in Chapter 2 shows the variation in temperature during seed replication in a common environment. Temperature varied from - 4 to 25 °C.

Figure 3.1 shows the initial percentage seedling emergence for seeds sown out for replication in a common environment. The populations are presented according to latitude, from south to north. Six populations failed to germinate and were excluded Figure 3.1. These were the populations from Gottingen (5) and Braunschweig (6) in Germany; from Milejowice (8) and Kobierzyce (9) in Poland; Weheldingen (10) in the Netherlands and Peldon (17) in England. It is interesting to note that these populations were all supplied by SAC Aberdeen and were collected a year earlier, in 1994. It was also clear from Figure 3.1 that for those seeds supplied by SAC Aberdeen that germinated (unshaded bars), initial seedling emergence tended to be low, with the exception of the populations from Badel (2), Bzouidan (3) and Stamfordham (32). It should also be noted that initial charactersitics for the Edinburgh population were not collected as replicate pots for this population were established a month later. Overall significant variation in emergence was recorded between populations, from 3 % for the Bonn population (4) to 100 % for the Bristol 2 population (16). It is apparent that there was no trend in percentage seedling emergence according to latitude.

Figure 3.2 shows the days from sowing to seedling emergence for seeds sown out for replication in a common environment. The number of days between sowing and emergence varied between 15 and 41 days for different pots, with a mode of 19 days. It was noted that there appeared to be a relationship between mean % emergence and time to emergence, with populations with the lowest percentage emergence also being the slowest to emerge. This is shown in Figure 3.3. Again there was no apparent geographical pattern in time between sowing and emergence.

Figure 3.4 shows the number of days from sowing to first flowering of the different populations of *G. aparine* grown in a common environment. The number of days between

sowing and flowering varied between 131 and 193 days for different pots. The majority of populations flowered between 160 and 170 days after sowing. The population from Caithness (38) was a notable exception, with first flowering recorded after 185 days. Again there was no apparent geographical pattern in time between sowing and flowering

Code	Country	Nearest town	Latitude	Longitude
1	Italy	Bologna	44° 30′ N	11° 20′ E
2	Switzerland	Badel	47° 33′ N	7° 36′ E
3	France	Bzouidan	-	-
4	Germany	Bonn	50° 44′ N	7° 06′ E
5	Germany	Gottingen	51° 32′ N	9° 57′ E
6	Germany	Braunschweig	52° 15′ N	10° 30′ E
7	Germany	Salzdahlum	-	-
8	Poland	Milejowice	-	-
9	Poland	Kobierzyce	-	-
10	Netherlands	Weheldingen	-	-
11	England	Winchester 1	51° 04′ N	1° 19′ W
12	England	Winchester 2	51° 04′ N	1° 19′ W
13	England	Ashford 1	51° 11′ N	0° 56′ E
14	England	Ashford 2	51° 11′ N	0° 56′ E
15	England	Bristol 1	51° 26′ N	2° 44′ W
16	England	Bristol 2	51° 26′ N	2° 44′ W
17	England	Peldon	51° 50′ N	0° 55′ E
18	England	Harpenden	51° 49′ N	0° 22′ W
19	England	Cambridge	52° 15′ N	0° 02′ W
20	England	Ely	52° 24′ N	0° 16′ E
21	England	Loddington	52° 34′ N	0° 52′ W
22	England	Aylsham	52° 50′ N	1° 15′ E
23	England	Wymondham	52° 34′ N	1° 07′ E
24	England	Hereford	52° 09′ N	2° 41′ W
25	England	Wellesbourne	52° 19′ N	1° 35′ W
26	England	Drayton	52° 12′ N	1° 46′ W
27	England	Meden Vale 1	53° 13′ N	1° 08′ W
28	England *	Meden Vale 2	53° 13′ N	1° 08′ W
29	England	Malton	54° 08′ N	0° 48′ W
30	Northern Ireland	Waringstown	54° 26′ N	6° 18′ W
31	Northern Ireland	Hillsborough	54° 28′ N	6° 05′ W
32	England	Stamfordham	55° 02′ N	1° 53′ W
33	Scotland	St Boswells	55° 34′ N	2° 38′ W
34	Scotland	Edinburgh	55° 52′ N	3° 12′ W
35	Scotland *	Perth 1	56° 23′ N	3° 19′ W
36	Scotland	Perth 2	56° 25′ N	3° 37′ W
37	Scotland	Aberdeen	57° 20′ N	2° 20′ W
38	Scotland	Thurso	58° 30′ N	3° 14′ W

Table 3.2: Locations and site descriptions for collected populations of *Galium aparine*.
* denotes populations collected from within 10 m of a field boundary.
- denotes missing latitude and longitude statistics.

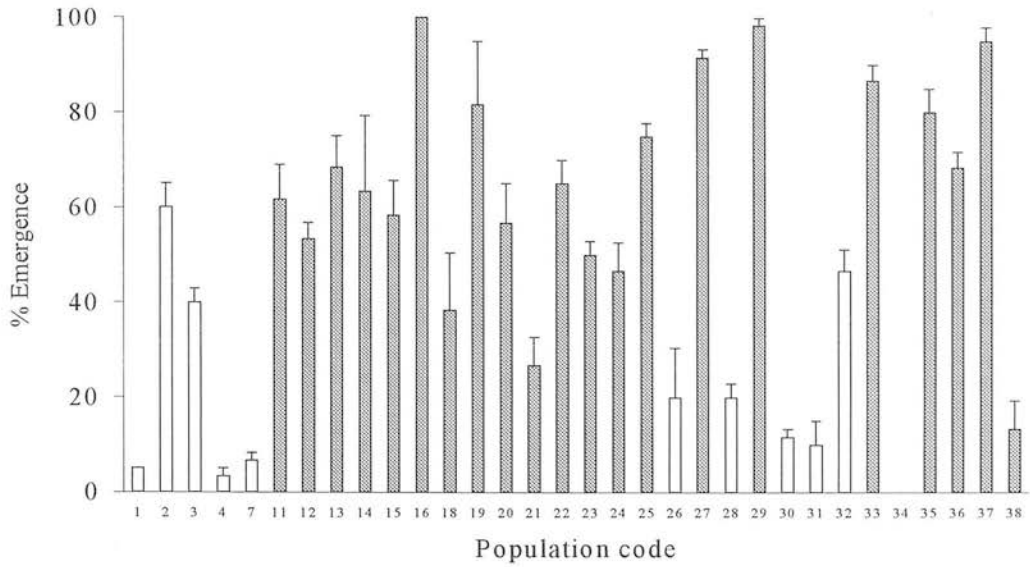


Figure 3.1: Initial percentage seedling emergence for seeds of *Galium aparine* sown out for replication in a common environment.
n = 3; 20 seeds sown per replicate; mean emergence ± 1 S.E.
Population codes as Table 3.2.
Shaded bars are for seed populations collected summer 1995.

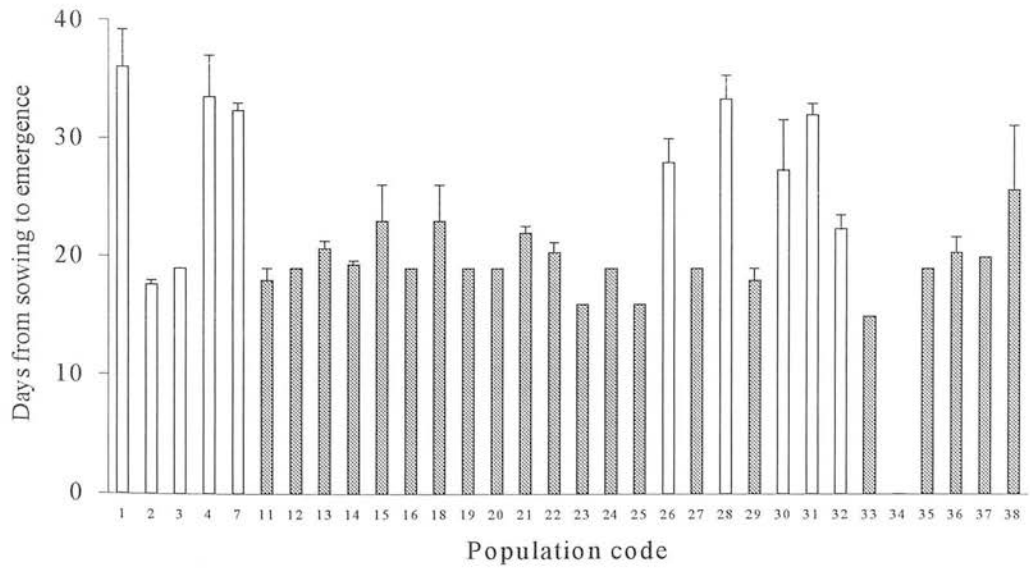


Figure 3.2: Days from sowing to first emergence for seeds of *Galium aparine* sown out for replication in a common environment.
Mean day ± 1 S.E. Other details as for Figure 3.1.

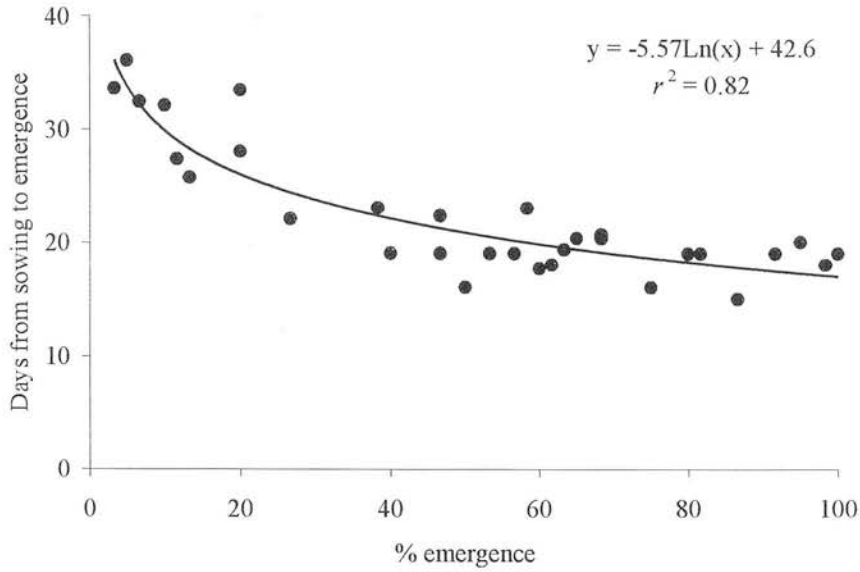


Figure 3.3: Relationship between mean percentage emergence and time from sowing to emergence for *Galium aparine.*

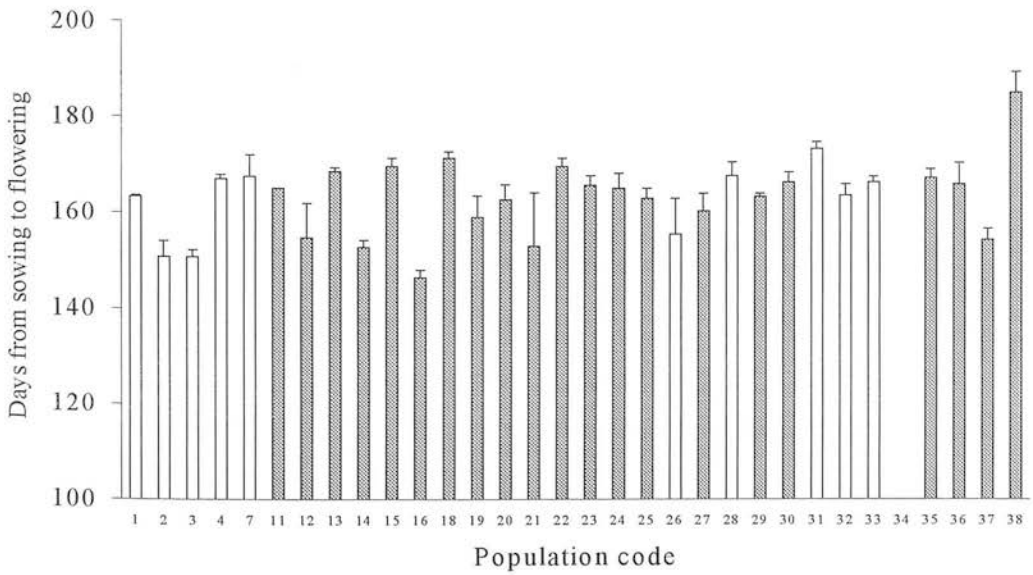


Figure 3.4: Days from sowing to first flowering for seeds of *Galium aparine* sown out for replication in a common environment.
Mean day ± 1 S.E. Other details as for Figure 3.1.

3.4.3. Screening for differences in seed characteristics

Figure 3.5 shows significant differences between populations for mean 100 seed weight. The number of populations surveyed was further reduced from 32 to 30 because the Ely (20) and St Boswells (33) populations failed to set seed. The heaviest seeds were from Waringstown (30) and the lightest were from Caithness (38).

Figure 3.6 shows the relationship between population seed diameter and population 100 seed weight. Seed diameter measurements were restricted to those populations that germinated to more than 10 % in the warm cabinet and for this reason the number of comparisons is restricted (see section 3.4.4. below). Figure 3.6 illustrates that for the populations that germinated, the majority of seeds were between 0.006 and 0.012 g in weight and had a diameter between 2.8 and 4.0 mm. The Waringstown (30) population was significantly larger in seed size. Again it is interesting to note that a linear relationship can describe the relationship between seed diameter and seed weight and that the two dimensions do not scale cubically as might be expected if the relationship between seed size and seed density remains constant.

Observations of seed colour show that the predominant seed colour tended to be brown (2), but that the seed produced by the population from Malton (29) and Perth 1 (35) tended to be lighter in colour than seed from the Aberdeen (37) population.

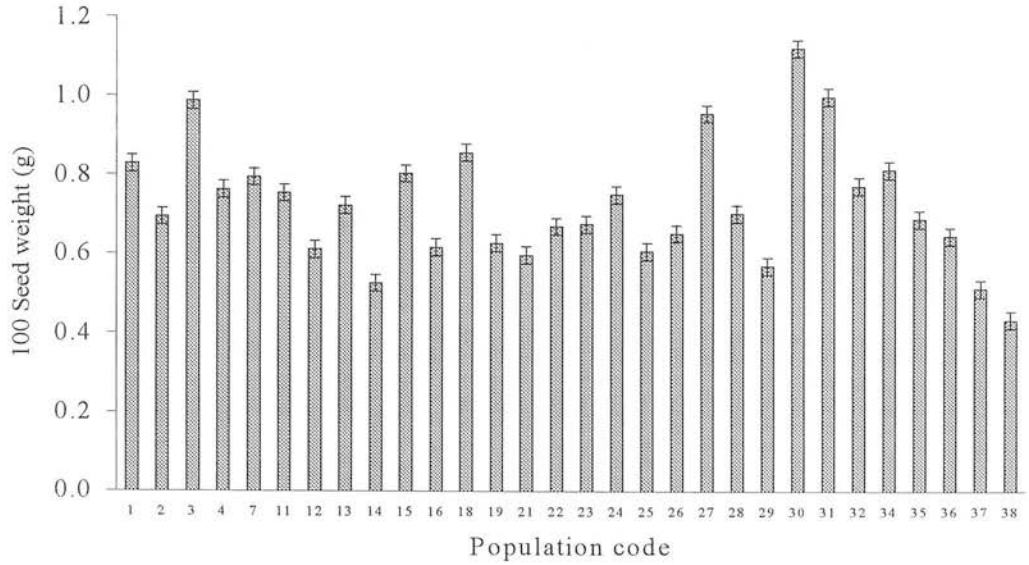


Figure 3.5: 100 seed weight for populations of *Galium aparine* produced in a common environment, August 1996.
n = 6; mean 100 seed weight ± 95% confidence interval.
Population codes as Table 3.2
$F_{(29, 150)} =$. $P < 0.001$

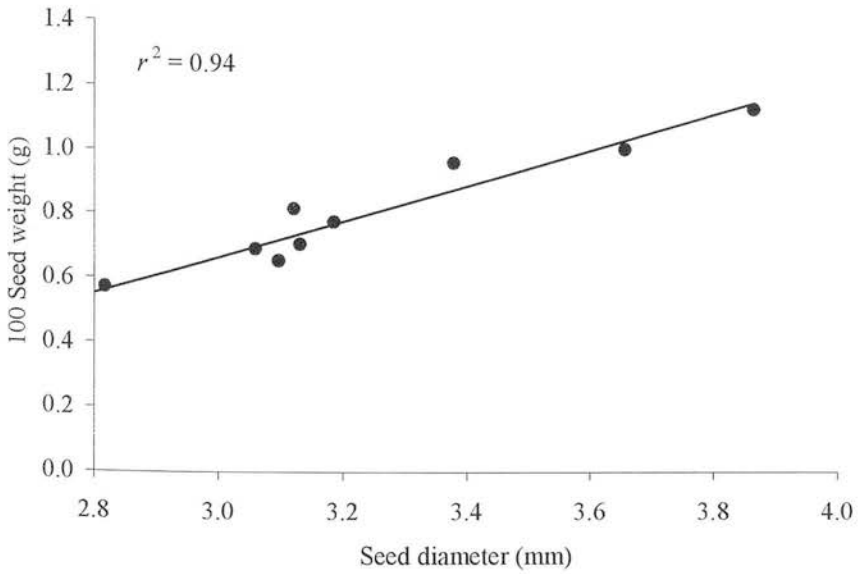


Figure 3.6: Mean seed diameter versus 100 seed weight for populations of *Galium aparine* produced in a common environment, August 1996.

3.4.4. Screening for differences in seed germination characteristics

Table 3.3 shows the actual percentage germination for each of populations in the two temperature regimes. A large number of populations failed to germinate or germinated to only low levels irrespective of temperature regime. Additionally it is clear that seed germination was low for all populations in the colder temperature regime. For this reason, further analysis by fitting Gompertz curves was restricted to cumulated seed germination data for populations with greater than 10 % germination in the warm temperature regime. It was noted that there was little correspondence between final percentage germination in the two temperature regimes and that the Caithness population (38), despite largely failing to germinate in the warmer temperature regime, germinated to > 15 % in the colder temperature regime.

Table 3.4 gives the population parameters derived from fitting the Gompertz curve to the cumulated counts of germinated seeds in the warm temperature regime. Table 3.5 gives a summary of the analysis of variance for the fitted Gompertz parameters according to population. The analysis showed that there were significant differences between populations for the parameters μ and for the calculated value for t_{50}. The Meden Vale 1 (27) population had the shortest time to inflexion (μ), whilst the Waringstown (30) population had the longest. The slow germination of the Meden Vale 2 (28) population was also reflected in the slow time to 50 % germination (t_{50}).

Figure 3.7 illustrates the fit of the Gompertz curve to the data and illustrates the different germination time courses for two contrasting populations.

Code	Country	Nearest town	20/15 °C %	S.E.	10/5 °C %	S.E.
1	Italy	Bologna	1.3	0.9	1.3	1.0
2	Switzerland	Badel	6.2	0.7	0	0
3	France	Bzouidan	0	0	0	0
4	Germany	Bonn	5.3	0.7	0	0
7	Germany	Salzdahlum	0.4	0.5	0	0
11	England	Winchester 1	1.8	0.5	0	0
12	England	Winchester 2	1.8	0.8	2.7	1.0
13	England	Ashford 1	0.4	0.5	0.7	0.7
14	England	Ashford 2	0.9	0.5	0	0
15	England	Bristol 1	0.9	0.7	0	0
16	England	Bristol 2	2.7	0.9	0	0
18	England	Harpenden	0.4	0.5	0	0
19	England	Cambridge	0	0	0	0
21	England	Loddington	2.2	0.5	0.7	0.7
22	England	Aylsham	1.3	0.9	0.7	0.7
23	England	Wymondham	8.4	0.8	0.7	0.7
24	England	Hereford	9.3	1.3	1.3	0
25	England	Wellesbourne	2.7	0.7	0	0
26	England	Drayton	52.9	2.2	5.3	1.4
27	England	Meden Vale 1	88.4	0.7	6.0	1.2
28	England	Meden Vale 2	87.6	1.9	6.0	2.1
29	England	Malton	55.6	1.0	1.3	0
30	Northern Ireland	Waringstown	56.9	1.8	8.0	1.7
31	Northern Ireland	Hillsborough	73.3	1.4	0	0
32	England	Stamfordham	90.7	1.6	1.3	0
34	Scotland	Edinburgh	72.4	1.9	0.7	0.7
35	Scotland	Perth 1	56.9	1.6	10.7	0
36	Scotland	Perth 2	2.2	1.0	1.3	1.0
37	Scotland	Aberdeen	83.6	1.8	2.0	1.2
38	Scotland	Thurso	0.4	0.5	17.3	1.4

Table 3.3: Actual final percentage germination for *Galium aparine* populations at 20/15 and 10/5 °C.
n = 3.

Code	Nearest town	β hr^{-1}	μ hr	γ	α	t_{50} hr
26	Drayton	0.013	280.2	63.4	-0.7	320.0
27	Meden Vale 1	0.015	184.4	93.8	-2.4	221.7
28	Meden Vale 2	0.010	278.0	102.9	-1.1	321.4
29	Malton	0.025	156.5	55.1	-0.5	173.4
30	Waringstown	0.029	184.9	49.1	-0.5	200.4
31	Hillsborough	0.015	283.8	88.2	-0.1	322.6
32	Stamfordham	0.015	233.7	100.5	-1.5	273.3
34	Edinburgh	0.012	306.9	97.6	-1.0	352.4
35	Perth 1	0.017	176.1	54.1	-0.8	199.2
37	Aberdeen	0.019	185.8	83.2	-1.8	208.0
	Mean	0.017	227.0	78.8	-1.0	259.2

Table 3.4: Mean parameters from fitting the Gompertz curve to cumulated counts of *Galium aparine* seeds germinating at 20/15 °C.
n = 3.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	9	2.28	-	5.96	***	5.82	***
Residual	19						

(1 missing value)

Table 3.5: Summary of the analysis of variance for the differences between populations for fitted Gompertz parameters at 20/15 °C. Data for 10/5 °C excluded from analysis because of low levels of germination.
F ratios with significance as *** P < 0.001; ** P < 0.010 and * P < 0.050.

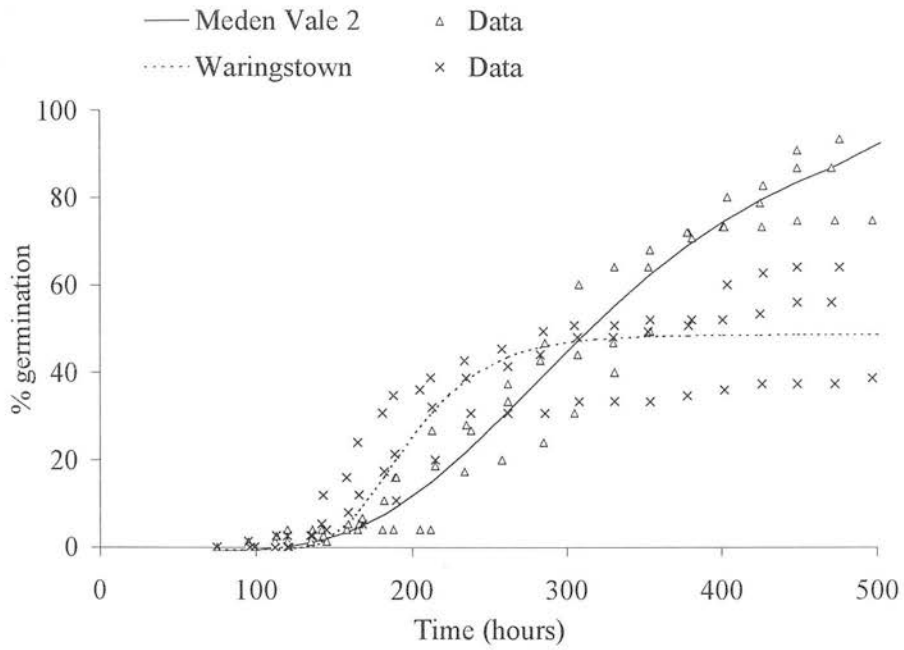


Figure 3.7: Fitted Gompertz curve compared with actual germination time course for two contrasting populations of *Galium aparine* at 20/15 °C.
n = 3.

3.4.5. Screening for differences in seedling growth characteristics

Table 3.6 shows the mean population statistics from time of sowing (germination) to emergence and to one whorl in controlled conditions. Differences between populations in time from sowing to emergence and to one whorl were significant. Overall seedling emergence occurred between two and 32 days after sowing and the first whorls were then recorded after 12 to 40 days. Assuming a base temperature of 0 °C (see Chapter 7), this corresponded to a thermal time requirement of 36 to 576 degree days for cotyledon emergence and 216 to 720 degree days for emergence of the first whorl. The Hillsborough (31) population was notably slower to emerge in contrast to the Malton (29) and Aberdeen (37) populations. Differences in time taken to emerge were not consistent with time from sowing to first whorl. The Meden Vale populations (27 & 28) tended to be fastest to grow to the 1 whorl stage and the Aberdeen (37) population tended to be slowest.

Figure 3.8 and Figure 3.9 show the mean seedling height and width statistics for the populations. There were significant differences between populations for each of these statistics at the 1 whorl stage. The Waringstown (30) and Meden Vale 1 (27) populations

were the tallest and the latter was also the widest at first whorl. In contrast, the Edinburgh (34) population was the shortest and narrowest.

Figure 3.10 shows the difference in the number of leaflets recorded in the first whorl which varied between four and six. The Hillsborough (31) population tended to six, whilst the Meden Vale 2 (28) and Edinburgh (34) populations tended to four.

Figure 3.11 shows differences between populations in dry weight recorded at one whorl. The Waringstown (30) and Meden Vale 1 (27) populations produced among the heaviest seedling dry weights and the Stamfordham (34) population produced seedlings of the lowest dry weights.

Figure 3.12 shows differences between populations in seedling relative growth rate for dry weight from sowing to first whorl. The relative growth rate for dry weight of seedlings from the Aberdeen (37) and Malton (29) populations tended to be high, in contrast with the slow growth rates recorded for the Hillsborough (31) population.

Code	Nearest town	t_1 (days)	t_2 (days)
26	Drayton	10.87	17.86
27	Meden Vale 1	10.55	16.90
28	Meden Vale 2	10.74	16.34
29	Malton	8.46	18.41
30	Waringstown	11.35	18.34
31	Hillsborough	11.82	18.52
32	Stamfordham	11.38	19.14
34	Edinburgh	10.88	19.09
35	Perth 1	9.64	17.59
37	Aberdeen	8.27	19.40
	Mean	10.40	17.67
	F	6.00	5.00
	df	[9, 632]	[9, 632]
	P	***	***

Table 3.6: Mean population seedling statistics for time from sowing (germination) to emergence (t_1) and to one whorl (t_2) for 10 UK populations of *Galium aparine* n = >50. *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$.

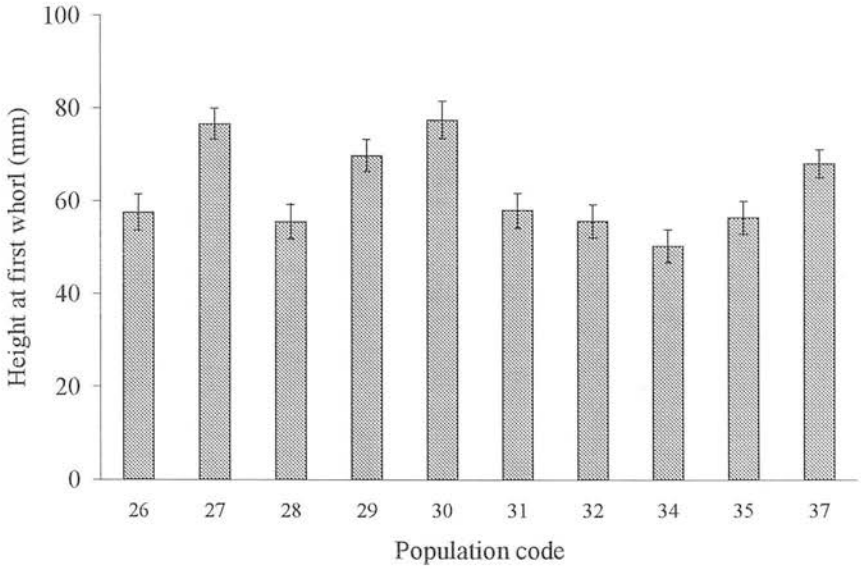


Figure 3.8: Seedling height at one whorl (h_1) for *Galium aparine* populations.
n >= 50; mean height ± 95% confidence interval. Population codes as Table 3.2.
$F_{(9, 632)} = 32.0$. $P < 0.001$.

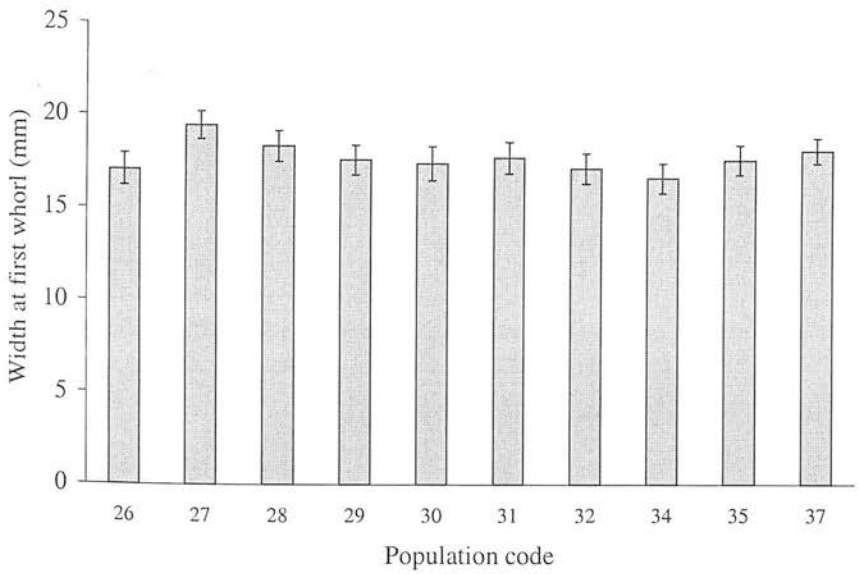


Figure 3.9: Seedling width across first whorl at one whorl (w_1) for *Galium aparine* populations.
Mean width ± 95% confidence interval. Other details as for Figure 3.8.
$F_{(9, 632)} = 5.0$. $P < 0.001$.

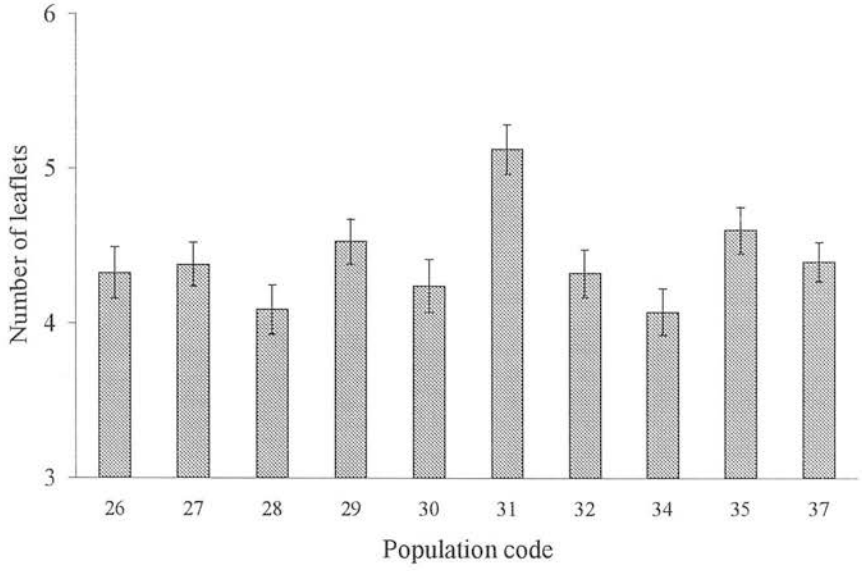


Figure 3.10: Number of leaflets in first whorl at one whorl (n_1) for *Galium aparine* populations.
Mean number of leaflets ± 95% confidence interval. Other details as Figure 3.8.
$F_{(9, 632)} = 16.7$. $P < 0.001$.

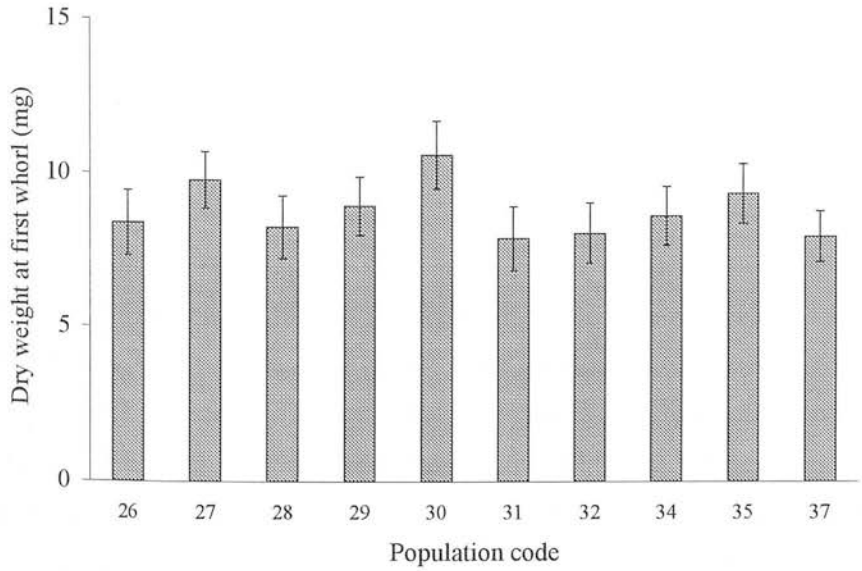


Figure 3.11: Seedling dry weight at on whorl (d_1) for *Galium aparine* populations.
Mean width ± 95% confidence interval. Other details as Figure 3.8.
$F_{(9, 632)} = 3.27$. $P < 0.001$.

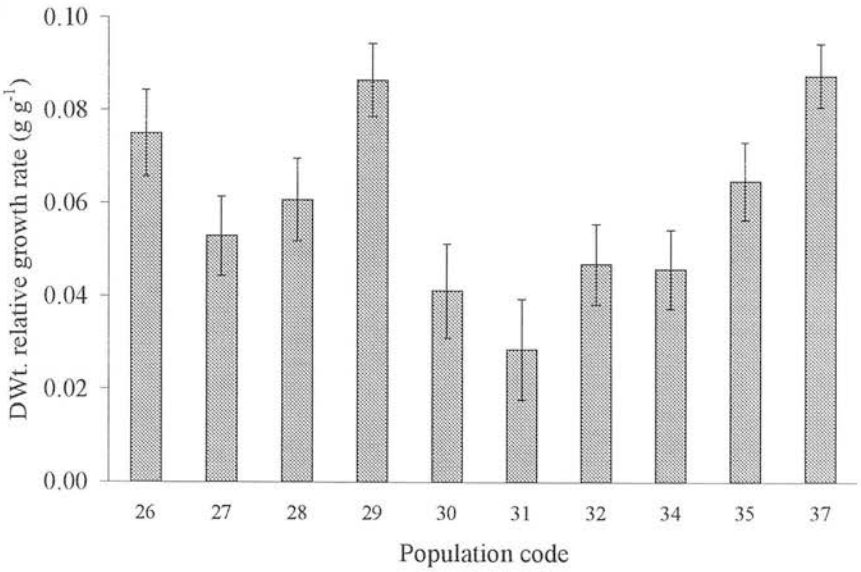


Figure 3.12: Seedling relative growth rates to one whorl (r_{d1}) for *Galium aparine* populations.
Mean relative growth rate ± 95% confidence interval. Other details as Figure 3.8.
$F_{(9, 557)} = 26.5$. $P < 0.001$.

3.4.6. Prediction of dry weight and relative growth rates from other seedling characteristics

Partial least squares regression of the seedling growth statistics was applied with seedling dry weight and relative growth rates for dry weight as the dependent variables. The results are shown in Table 3.7. Cross validation showed that only factor 1 was relevant for prediction. The analysis showed that for seedling dry weight at one whorl, seedling height at one whorl was the variable most closely related to factor 1. For seedling relative growth rate to one whorl, time from sowing to emergence and from sowing to one whorl, together with seed diameter were associated with factor 1. The high percentage total variance explained by factor 1, suggested that measurements of seedling height at one whorl could be used to estimate seedling dry weight at one whorl, and seed size in combination with the timing of seedling developmental events could be used to estimate seedling relative growth rate without need for destructive measurements.

Dependent variable	Dry weight (d_1)	Relative growth rate (r_{d1})
% total variance of dependent variable explained by factor 1	66.3	87.3
Independent variable	% total variance explained by factor 1	
Seed diameter (sd)	18.8	71.6
Seed colour	18.0	50.2
Radicle length	31.3	1.7
Number of leaflets in first whorl	0.0	3.1
Height at one whorl	85.4	4.2
Width across whorl at first whorl	27.2	2.4
Time from sowing to emergence (t1)	1.0	92.0
Time from sowing to first whorl (t2)	0.1	94.1

Table 3.7: Partial least squares regression analysis of variance for *Galium aparine* seedling characteristics using (a) seedling dry weight at one whorl (d_1) and (b) seedling relative growth rate to one whorl (r_{d1}) as dependent variables.

3.4.7. Selection of contrasting populations

Figure 3.13 shows the similarity matrix calculated from mean population statistics for seed size, seed colour, seed germination and seedling growth statistics (details in Table 3.1). The similarity matrix shows that on the basis of the data entered, the populations from Malton (29) and Edinburgh (34) were the most dissimilar (48 %) and that the populations from the Malton (29) and Perth (35) were the most similar (95 %). It is interesting to note that the two Meden Vale populations (1 and 2) were 83 % similar.

P	26	27	28	29	30	31	32	34	35	37
26	----									
27	75	----								
28	91	83	----							
29	74	75	68	----						
30	69	78	65	76	----					
31	81	72	88	50	66	----				
32	89	86	94	68	70	88	----			
34	91	69	90	48	53	84	92	----		
35	84	82	79	95	81	66	80	63	----	
37	82	85	81	91	64	60	82	68	89	----

Figure 3.13: Similarity matrix for *Galium aparine* populations calculated from mean seed size and colour, seed germination and seedling growth statistics.
Statistics entered as Table 3.1.
Population codes as Table 3.2.

Furthest neighbour cluster analysis based on the similarity matrix in Figure 3.13 is shown in Figure 3.14. Cluster analysis identified the Malton (29), Perthshire (35), Aberdeen (37), Meden Vale 1 (27) and Waringstown (30) populations as atypical from largest cluster. The position of the Malton (29) populations as an 'extreme' population was validated by ranking the populations for each statistic and summing the number of top five and bottom five rankings. However the next highest ranking population was Hillsborough (31). Inspection of the characters by which the populations differed showed that these populations represented different 'extremes' so the Hillsborough population was selected as the other 'extreme' population. The Stamfordham (32) population was lowest scoring and was therefore selected as the most 'typical' population.

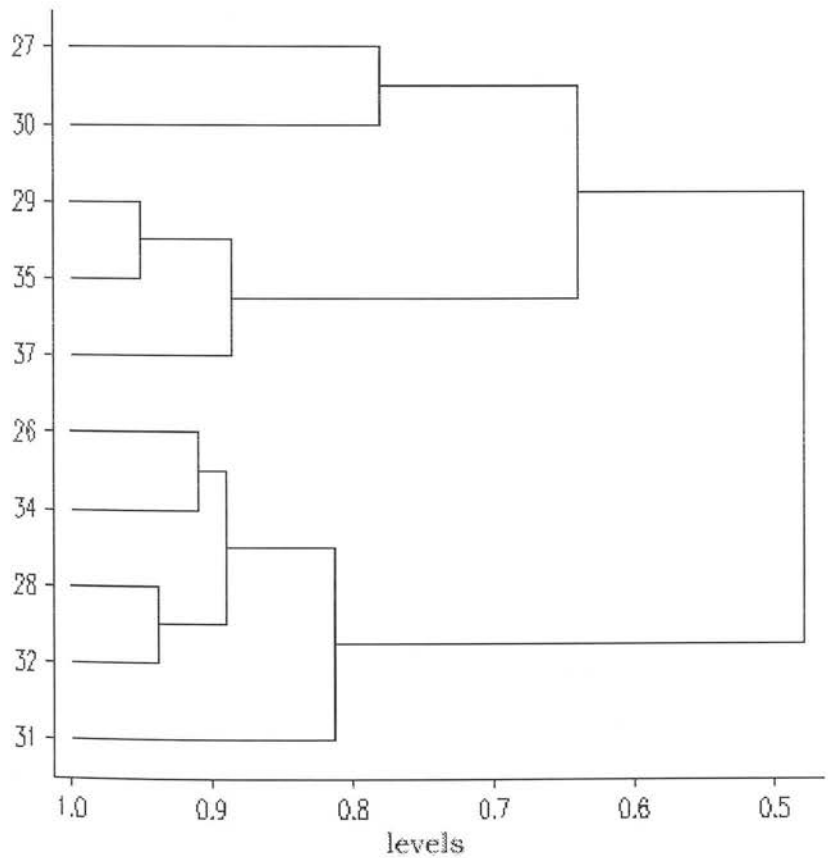


Figure 3.14: Furthest neighbour cluster analysis of *Galium aparine* populations according to seed germination and seedling growth statistics.
Population codes as Table 3.2.

3.5. Discussion

3.5.1. Seed collection and sampling strategy

Table 3.2 shows that seed was collected from a wide range of locations across the UK and Europe. In retrospect, it was probably unwise to include the additional seed populations about which less information was known, especially regarding storage conditions prior to receipt.

3.5.2. Seed replication and initial observations

It is suggested that the six populations that failed to emerge were non-viable (although this was not tested directly). Temperature variation over the period of seed replication would suggest that suitable conditions for seed germination should have been fulfilled at some point between October 1995 and August 1996. Pots were kept watered and the soil surface was kept clear. It is not clear why seed from these six populations failed to germinate, although low emergence for the other seed populations from SAC Aberdeen might suggest that the seeds had previously been stored in less than ideal conditions. These seeds were also older and *G. aparine* seeds have been shown to lose viability progressively over time (Grime, Hodgson & Hunt, 1988; Brenchley & Warington, 1930, 1933).

It was clear from initial observations of seedling emergence and flowering times, that populations differed, although as these seed had been produced in different environments, the extent to which this reflected actual genetic differences could not be assessed. There were no apparent geographic patterns in emergence time or percentage emergence, at least not that could be identified against the background variation in behaviour attributable to seed age and seed source. However, it was noted that the most northerly population was the latest population to flower (possibly reflecting a difference in day length requirements) and that flowering times for the Ely (20) and St Boswells (33) populations were not atypical, despite their subsequent failure to set seed.

It was also interesting to note that there was generally poor correlation in seedling emergence statistics between populations collected on the same date at nearby locations (e.g. Winchester 1 (11) and 2 (12) and Bristol 1 (15) and 2 (16)), suggesting that local populations were no more likely to be similar than more distant populations. It was also noteworthy that again there was a generally close correlation between percentage emergence and time to

emergence. Populations with high percentage emergence also emerged earlier than populations with low emergence.

3.5.3. Differences in physical seed characteristics

It was not clear why the Ely (20) and St Boswells (33) populations failed to set seed. It is possible that these populations were obligate out-crossing and that covering with muslin to prevented cross-pollination between populations, inhibiting seed set. However *G. aparine* is normally described as self pollinating (Malik & van den Born, 1988) and self incompatible populations have not previously been described.

Previously quoted mean seed weights have varied from 7.25 mg (Grime, Hodgson & Hunt., 1988) to between 3 and 6 mg (Malik & van den Born, 1988) to 11 mg (Ligneau & Watt, 1995). The range of mean seed weights recorded in this study was between 4.4 to 11.2 mg, with an average of 7.3 $\pm$ 0.1 mg. The Northern Ireland populations (30 & 31) in particular were larger than those previously recorded. Seed weight and seed diameter differed significantly between populations. Generally there were no consistent patterns in seed size according to location, although it was noted that from North Yorkshire northwards there appeared to be a trend in decreasing average seed weight. It has been suggested that the shorter growing seasons might select for smaller seeds in order to maximise levels of seed production from a relatively smaller pool of accumulated resources. However it is clear that this requires further investigation.

3.5.4. Differences in seed germination characteristics

It was not clear why such a high proportion of the *G. aparine* seed populations failed to germinate in both of the two test temperature regimes. It was noteworthy that those populations that did germinate were almost exclusively populations that originated from sites relatively close to the Edinburgh site, where the seeds were replicated and this might suggest that these populations were better adapted to the prevailing local climate. However the Drayton population (26) and Perth 2 (36) populations were exceptions to this general pattern, with relatively high germination occurring for the Drayton (26) population and minimal germination for the Perth 2 (36) population. Tetrazolium tests (methods described in Ellis, Hong & Roberts, 1985) were conducted on a sub-set of the *G. aparine* populations that failed to germinate to establish the extent of seed viability. These showed that the seeds were largely viable (results not presented) and suggested that the seed populations were

expressing different levels of dormancy. As an alternative explanation, it was suggested that the relatively high irradiances were perhaps inhibiting seed germination as Malik & van den Born (1988) reported that *G. aparine* germination was inhibited by light and Grime *et al.* (1981) reported that germination percentage was maximised under shade conditions (4.5 μmol m^{-2} s^{-1} (converted from 0.97 W m^{-2} following Hall *et al.*, 1993)). Additional experiments in complete darkness (with identical temperature conditions) recorded similarly low levels of germination for populations that largely failed to germinate under the standard light treatment (results not presented). It therefore remains unclear why such a large number of populations failed to germinate and it should be remembered that all subsequent consideration of population differences between *G. aparine* populations are restricted to populations drawn from a relatively small area. It is likely that these populations were more similar to each other than to the other populations that failed to germinate.

For those seeds that germinated, the effect of temperature on the proportion of seeds germinating and the synchronicity of germination mirrored the patterns of autumn and spring emergence of *G. aparine* in the field. Colder temperatures limited seed germination, although the Caithness (38) population was a notable exception, germinating exclusively in the colder regime, albeit still at a relatively low level. It is possible that this reflects lower temperature requirements for germination of this the most northerly population.

It was noteworthy that the significantly larger seeds of the Waringstown (30) population germinated to the highest final percentage at the slowest rate.

3.5.5. Differences in seedling growth characteristics

The Waringstown (30) population also tended to produce amongst the largest and heaviest seedlings. For other populations the relationship between mean seed size and seedling size statistics were less pronounced. Seed size however was closely associated with seedling growth rate, with smaller seed populations growing at a relatively faster rate.

Grime *et al.* (1988) quote seedling relative growth rates ranging from 0.14 and 0.20 day^{-1} for the five weeks from emergence. Similarly Grime & Hunt (1975) quote seedling relative growth rates of 0.17 day^{-1} and Fitter and Peat (1994) have reported rates of 0.216 day^{-1}. These values were all greater than the values calculated for seedling growth from emergence to one whorl in this investigation and this may be related to the relatively short time scale

involved, with growth rate possibly accelerating as seedlings become more established beyond the first whorl.

Again the significant variation between populations of *G. aparine* in germination and seedling growth can largely be attributed to genetic differences between populations. The particular differences that were recorded were undoubtedly the result of complex interactions between the maternal and test environments and the genetic structure of the populations and it should be remembered that different responses would be expected if the seeds had been produced in a different maternal environment or if the seedlings had been grown under different conditions. The magnitude of differences in germination and seedling growth characteristics observed in this study suggest that future studies aimed at describing the ecology of *G. aparine* need to consider the extent of inter-population variability.

3.5.6. Difficulties in predicting seedling dry weight and relative growth rates

It was clear that there was substantial potential in using seedling height to estimate seedling dry weight at one whorl. This was not altogether surprising as the first whorl of leaves comprised only a small fraction of total seedling weight at one whorl and differences between seedlings were more obviously related to seed height compared to differences in cotyledon size. It should be noted that the differences between populations were restricted to above ground seedling dry weight and no measurements were made of seedling root dry weight. It is possible that the differences observed are differences related to the partitioning of dry weight and that total seedling dry weight may not differ between populations. Clearly this needs further investigation.

Seedling relative growth rate could largely be predicted by seed weight and observation for time from sowing to emergence and to one whorl. This is again not altogether surprising as time was included within the calculation of seedling relative growth rate and seed size is related to seedling size and seedling vigour (Harper & Ogden, 1970: Egli, TeKrony & Wiralaga, 1990).

3.5.7. Final selection of contrasting populations

Populations were shown to differ markedly, and at a higher level than that recorded for the screening of *S. media* populations. The two methods used were complementary and it was

reassuring that both identified the Malton (29) and Hillsborough (31) as contrasting populations and the Stamfordham (32) as 'typical'.

3.6. Conclusions

In common with the conclusions of Chapter 2, it was shown that there was significant variation between populations in germination and seedling growth and these differences could largely be attributed to genetic differences. This was clearly illustrated for *G. aparine* following seed replication in a common environment. It was however noted that a high proportion of the seed populations were excluded from the analysis, both following failure to germinate and set seed in the common environment and following failure to germinate or low levels of germination in the two test environments. This was particularly marked for the colder temperature environment and therefore analysis was restricted to seeds germinated in the warmer environment. It was noted that this then largely restricted population selection to populations drawn from areas with similar climates to the common environment in which the seeds were replicated.

Chapter 4. Introduction to modelling

4.1. Summary

Modelling principles, terminology and the general practicalities of constructing weed population models are introduced. Ecophysiological models of weed seedling emergence are then reviewed in detail in order to identify the strengths and weaknesses of existing models. The utility of these quantitative models in the synthesis of seed dormancy, germination and seedling emergence responses to environmental conditions is recognised and a framework is established for integrating understanding of the seed ecology of *Stellaria media* and *Galium aparine*. Existing data with potential use in modelling seed behaviour for these two species are identified and reviewed. This leads to the identification of new information that is required to formulate models of seed dormancy and germination for *S. media* and *G. aparine*. These include quantitative investigations into seasonal dormancy changes in *S. media*; the combined effects of temperature and water potential on seed germination; the effects of light and nitrate on germination of *S. media* and assessment of extent of inherent and environmentally determined variability within and between populations of *S. media* and *G. aparine*.

4.2. Introduction

Poor understanding of the relationship between environmental factors and the biological processes that occur between seed set and seedling establishment was identified in Chapter 1 as a major problem when predicting the distribution and abundance of weeds. Chapter 1 also noted that the most successful predictions of weed seedling emergence have been made using explicit models based on a description of biological processes (Vleeshouwers, 1997). As such, this thesis has adopted a dual approach to advance understanding of seed dormancy and germination for *S. media* and *G. aparine*. This centres on linking targeted experimental studies with the development of models of seed dormancy and seed germination. The models were developed to incorporate existing experimental data and help to direct new experimental work. A major concern in this modelling exercise was also the need to include realistic representation of variation within species, some extent of which was illustrated in the previous two chapters.

Interest in models of weed population dynamics increased markedly during the 1990's, largely as a result of a perceived need to integrate experimental results and increase understanding of these complex dynamics in order to improve decision making related to weed control. Models have varied widely in formulation, scale and complexity. In order to review these differences and identify the most appropriate type of model for development in this thesis, this chapter begins with a summary of model terminology and broad differences in formulation. The chapter then reviews existing models developed to summarise the interactions between environmental factors and the biological processes related to weed seedling emergence. This is then followed by a review of the published ecological data for *Stellaria media* and *Galium aparine* with the aim of identifying research needs for model development.

Patterns of weed seedling emergence result from the reaction of individual seeds to the various environmental factors that effect seed dormancy and seed germination. When formulating models, our interest is in the effect of such factors at the population scale with the objective of understanding the consequences of different management practices. For this reason, this chapter will concern itself solely with the development and application of population models.

4.3. Review of model terminology and population models

The aim of population modelling is to scale up from individual behaviour to that of the population. There are essentially two scales at which population models can be formulated (van Gardingen, Curran & Foody, 1997). Most commonly, changes in population size and structure are defined at the level of the whole population. This contrasts with models of individual behaviour in which the aggregate behaviour of explicitly modelled individuals defines changes in population size and structure. Typically it is hoped that models defined at the population level capture this aggregate behaviour.

In formulating such population models, a number of further distinctions can be drawn.

4.3.1. Quantitative versus qualitative

The first distinction is between quantitative and qualitative models. Quantitative population models assign a specific numerical value to the population size or change in population size, which may correspond to individual births or deaths. These models are often used

predictively. In contrast, qualitative models may describe the size of a population in terms of categories, such as large or small, and can therefore only crudely describe changes in population size. As such these models are often considered to have less value predictively and it is clear that difficulties may be encountered in trying to formulate a qualitative population model based on individuals. However, it should be noted that in some circumstances, where quantitative information is limited, these models can be useful in describing the processes that effect changes in population size and/or structure.

4.3.2. Discrete versus continuous time

Essentially all biological processes occur in continuous time. For example, an individual may die at any time and in many organisms reproduction can occur throughout the year. However, in some organisms, processes such as reproduction may be restricted to certain well-defined intervals and as such, it may be appropriate to model changes to the individual or the population in discrete time. For example, seed germination in some species occurs only in the spring and therefore it may be of interest to model only year to year changes in this population. In a model formulated in discrete time with an annual time step, the population in the next year, N_{t+1} is a function, f of N_t, the population in the current year, t.

$$N_{t+1} = f(N_t) \tag{4.1}$$

It is evident that equation 4.1 describes or predicts the population at specific points in time. In contrast, a model formulated in continuous time defines the population size $N(t)$ at every point in time. In a continuous time formulation of a deterministic model, this is typically achieved by defining the rate of change, r, in $N(t)$ as shown in equation 4.2.

$$dN/dt = r(N(t)) \tag{4.2}$$

The population at time t is calculated from the initial population size by integration of equation 4.2. This integration is often achieved numerically.

4.3.3. Deterministic versus stochastic models

The distinction between deterministic and stochastic models is also important. Deterministic models describe changes to the individual or the population that are determined with

certainty from current and/or past individual status or state of the population. Equations 4.1 and 4.2 describe deterministic population-level models in discrete and continuous time respectively and in both cases, future population size is predicted with certainty from current population size. In contrast, stochastic models only define a set of possible future population sizes and the probabilities of obtaining them. Therefore if the initial population size is $N(0)$, a stochastic model typically predicts a probability distribution $p(N(t) \mid N(0))$ for the population size $N(t)$ at time t, given $N(0)$. A common approach to formulating stochastic models is to define the probabilities of events, such as births and deaths either at the population or the individual level. Stochasticity can be incorporated in deterministic models by randomly picking parameter values in multiple model runs, or by using stochastic time series for model inputs.

4.3.4. Model fitting statistics

Model fitting involves estimating the model parameters and calculating confidence intervals for each parameter. From a statistical viewpoint, appropriate fitting can only be achieved with stochastic models. For example, in stochastic models parameter estimates can be derived by the maximum likelihood method (section 2.3.3.2) and this allows calculation of confidence intervals and correlation between parameters.

In deterministic models, parameter estimates can simply be obtained by minimising the sum of squares between the model prediction and observed values. However in order to obtain confidence intervals, stochasticity is typically introduced by assuming that the error terms (differences between pairs of observed and expected values) are normally distributed and that successive values are not correlated. Since these assumptions are often difficult to justify, confidence intervals are not always given.

A frequently used and useful summary of model goodness of fit is the r^2 statistic. This is calculated as the fractional increase in variability (as measured by sum of squares) explained by the model over and above that explained by the mean of the data. However, it should be noted that the use of r^2 values to compare models with different numbers of parameters should be treated with caution, as model goodness of fit can be improved by simply adding more parameters.

4.3.5. Model validation

Model validation involves testing the model on new data (i.e. data not used in model fitting). Different models may then be compared by their ability to predict this new data. It is an important stage that can lead to model refinement and/or enhancement of model credibility.

4.4. Review of weed population models

Models that have been specifically developed to demonstrate weed population dynamics have varied in formulation. However for a review of these models, formulation was considered of secondary importance compared with the extent of biological realism incorporated.

4.4.1. Single stage models

Single stage or annual population growth models are the simplest form of weed population model. In these models an annual population growth rate (λ) is defined by assessing the difference in weed population size in discrete time at yearly intervals. This annual population growth rate is then used to calculate further changes in population size, assuming that conditions are constant from year to year. Selman (1970) and Mortimer (1987) have both described deterministic models of this type for *Avena fatua*. Mortimer (1987) additionally incorporated density dependent changes in the annual population growth rate. This adaptation reflected the main problem commonly encountered with such models, in that the annual population growth rates tend to be specific to a particular cropping system, soil type and climate. The lack of generality and need for site specific monitoring in models such as those developed by Selman (1970) and Mortimer (1987) limits their usefulness and predictive value.

To summarise, there are three main objections to this approach. Firstly these models by definition, do not predict population levels during the year. Secondly environmental factors are not normally taken into account, although modifications can be made to the population growth rate, for example using regression techniques. However, the results of such models tend to be difficult to interpret. This last point identifies the third objection, namely that it is difficult to incorporate biological understanding into these models as they do not explicitly model separate stages in seed to seedling development and the various stage-specific environmental interactions.

4.4.2. Multi-stage models

Multi-stage models are based on the life cycle of the species. The life cycle is split up into distinct stages and difference (discrete time) or differential (continuous time) equations are used to define transitions between stages. On the simplest level, weed populations are divided up into seed, seedling and seed producing plant stages. The future number of seeds is determined by rates of mortality for seeds and seedlings, emergence from seeds to seedlings and the number of seeds produced per surviving plant. These simple model components tend to be formulated in discrete time with time steps often defined at yearly intervals. Such models have been developed for a number of annual weed species that emerge, develop and die synchronously (e.g. Aarts, 1986 for *Galium aparine*). For perennial weeds and weeds with variable behaviour, this approach can be adapted to produce more complex models by dividing up the different stages into groups that differ in age (e.g. newly produced or old seed in the soil seedbank) or location (e.g. surface or buried seedbank). In such models, transitions are often defined in matrices and therefore these may be termed matrix models (e.g. Sarukhan & Gadgil, 1974 for *Ranunculus repens*; Law, 1983 for *Poa annua*).

Whilst these models are more biologically plausible, the simple examples detailed above again define transition rates between stages that do not explicitly depend on environmental conditions. Therefore for each new environment, new transitions rates would need to be estimated. If we are concerned with the actual biology of the weed species and how it interacts with the immediate local environment, the transition rates need to explicitly depend on environmental conditions.

4.4.3. Multi-stage ecophysiological models

In order to develop weed population models that can be useful in defining short-term changes in weed population size and structure according to environmental variation, it is necessary to adopt an ecophysiological or semi-mechanistic approach to modelling. Ecophysiological models define transition rates between different stages in the plant life cycle according to biological processes that are dependent on environmental conditions. In comparison with single stage models and multi-stage models defined for constant environments, it is clear that ecophysiological models may offer greater predictive value, especially in changing environments and on shorter time scales. Given that the stated aim of this thesis is to better understand the environmental determinants of weed seedling

emergence, the next section reviews existing ecophysiological models that describe weed seed dormancy, germination and pre-emergence growth.

4.5. Ecophysiological models of weed seedling emergence

A number of multi-stage ecophysiological models of weed seedling emergence have previously been developed. An ecophysiological approach to modelling *G. aparine* seedling emergence was adopted by van der Weide (1993), but no such models have been described for *S. media*.

It should also be noted that although this thesis focuses on weed seed dormancy and germination, models of weed seedling pre-emergence growth are also included in this review. Recent work, notably by Vleeshouwers (1997) has demonstrated the importance of distinguishing between germination and pre-emergence seedling growth in predictions of weed seedling emergence. This was also demonstrated by Fernandez-Quintanilla (1988), who showed that although germination of *Avena sterilis* was not significantly affected by increased burial depth, actual seedling emergence was markedly reduced. As such, unsuccessful emergence of germinated seeds may represent a large mortality factor for some weed species and may need to be included in models of weed seedling emergence. For simplicity, and to emphasise the need to distinguish seed dormancy from seed germination, the following review considers models of seed dormancy, seed germination and pre-emergence seedling growth separately.

4.5.1. Seed dormancy

Seasonal changes in weed seed dormancy have generally been modelled using the concept of dormancy proposed by Vegis (1964), and developed by Vleeshouwers (1997). In these models, changes in seed dormancy result in changes in the temperature requirements for germination. As dormancy is induced, the temperature range over which seeds can germinate narrows until the seeds are completely dormant and cannot germinate at any temperature. Conversely as dormancy is relieved, germination occurs over an increasingly wide range of temperatures. Changes in seed dormancy may also affect the level of seed germination.

Modelling of this phenomenon has been approached by two means. Spitters (1989) and van der Weide (1993) defined fixed calendar dates for seasonal changes in dormancy. In

contrast, Bouwmeester & Karssen (1992, 1993a, b, c) and Vleeshouwers (1997) developed models in which dormancy changed as a function of soil temperature. Bouwmeester & Karssen (1992, 1993a, b, c) modelled the seasonal changes in seed dormancy by the simultaneous action of a dormancy breaking and dormancy-inducing factor. The dormancy breaking factor was a cold sum, calculated as the time spent below a critical temperature, and the dormancy inducing factor was a heat sum, calculated by accumulating temperature during burial. These are effectively concepts of thermal time. Alternatively, Vleeshouwers (1997) modelled changes in seed dormancy by alternate periods of dormancy induction and dormancy relief, with the cycle shifting from dormancy relief to induction according to the level of a hypothetical factor X and in relation to defined upper and lower limits. Vleeshouwers (1997) argued that this more complex model was preferable because it was based on a theoretical model of the mechanisms of seed dormancy at the molecular level (Hilhorst, 1993, in Vleeshouwers, 1997).

Temperature dependence in the regulation of seed dormancy was also included in the model of Benech-Arnold *et al.*, (1990). Dormant seeds were classed as either 'inducible' or 'highly dormant'. Seeds from the 'inducible' fraction were able to germinate if subjected to alternating temperatures (with a specific amplitude and upper temperature limit), whilst seeds from the 'highly dormant' population remained permanently dormant.

4.5.2. Seed germination

In most germination models, seed germination is triggered by cultivation or exposure of the seeds to light (Spitters, 1989; van der Weide, 1993; Derkx & Karssen, 1993; Bouwmeester & Karssen, 1992, 1993a, b, c; Vleeshouwers, 1997). The relationship between dormancy level and field or test temperature then determines the extent of germination, though Bouwmeester & Karssen (1992, 1993a, b, c) additionally incorporate a modification related to the presence or absence of nitrate. Benech-Arnold *et al.* (1990) meanwhile, assumed that all seeds released from dormancy subsequently germinated.

Modelling of the onset of germination and subsequent germination rate has almost exclusively used the concept of thermal time, whereby temperature is accumulated above a base temperature and seeds germinate when a set value of thermal time is reached (Spitters, 1989; van der Weide, 1993; Benech-Arnold *et al.* 1990). However in these models, it should

be noted that it has been assumed that fluctuations in temperature *per se* do not affect the thermal time requirement.

In some cases the timing and extent of seed germination has been further modified by soil water potential, though notably not in the models of Bouwmeester & Karssen (1992, 1993a, b, c) or Vleeshouwers (1997). This has been achieved by a variety of methods, some of which have modelled changes only to germination rate, whilst others have included modifications to germination rate and final germination percentage. At the simplest level, Alm, Stoller & Wax (1993) presented an index model for germination rates according to soil water potential and temperature conditions. The model was defined with a germination rate in standard reference conditions (soil temperature 20 °C, 20 % temperature fluctuation, soil water potential 0 MPa). Germination under other environmental conditions was then calculated by multiplying the reference rate by environmental indices, ranging from 0 to 1, determined for alternative soil water potentials (-0.2, -0.4, -0.8 MPa) and temperatures (10, 15, 20 and 25 °C with temperature fluctuations of 0, 20, 40 and 60 %, producing a temperature range of 4 to 40 °C).

Spitters (1989) multiplied the maximum number of germinable seeds, time to first germination and germination rate as defined in conditions of optimum water supply by a factor that scaled linearly between 1 and 0, for values of soil water potential between 0 and – 1.5 MPa (range approximated from description of soil moisture tensions ranging from 2.0 to 4.2 pF). This had the effect of increasing time to first germination and decreasing final percentage germination and germination rate at low soil water potentials.

More recently weed population models have adopted the concept of hydrothermal time. This concept combines thermal time with an analogous model describing the effect of water potential on the timing of germination. This was first applied to crop plants by Gummerson (1986), but to date has only been applied to a single population of *Stellaria media* by Grundy (1997). For crop plants, the predictions of seedling emergence have been reasonably credible (Gummerson, 1986; Finch-Savage & Phelps, 1993), but the limited application of such methods to weeds has suggested that problems may occur where high levels of seed dormancy are present (Grundy, 1997).

It should be noted that seed dormancy has tended to be fed into these germination models as a single parameter, estimating the number of germinable seeds in a given set of

environmental conditions. As such, seed dormancy has been assumed not to affect the rate of germination. However recent experiments by Vleeshouwers (1998) on *Polygonum persicaria* showed that higher levels of seed dormancy were associated with slower germination rates.

4.5.3. Seedling emergence

The timing and extent of actual seedling emergence has been variously modelled. At the simplest level, Forcella (1992) developed a model that combined seed dormancy, seed germination and pre-emergence growth to predict emergence of *Chenopodium album* seedlings on the basis of accumulated temperatures (above a base temperature of 10 °C) in April.

Seedling emergence has also been equated with seed germination (Benech-Arnold *et al.*, 1990; Spitters, 1989), though Spitters (1989), incorporated an additional mortality factor to reduce the number of germinated seeds that emerged according to the extent of cycling between wet and dry conditions.

Harvey & Forcella (1993) introduced a delay between seed germination and seedling emergence according to a fixed rule that the time lag increased by one day for each additional centimetre of burial depth. This was used as a simple approximation and the authors acknowledged the need to relate seedling growth to temperature.

More explicitly, Alm *et al.* (1993) quantified the effect of temperature and water potential on hypocotyl elongation. Seedling emergence was then predicted in relation to seedling growth under standard reference conditions. In contrast van der Weide (1993) described rates of seedling emergence according to a Normal distribution function, characterised by a mean and standard deviation for thermal time requirements for germination. This was then modified by seed burial depth, soil resistance and a pre-emergence mortality factor to determine seedling emergence.

To date, Vleeshouwers (1997) has developed the most comprehensive model of seedling emergence. The model was developed through experimentation with three different weed species (*Chenopodium album, Polygonum persicaria* and *Spergula arvensis*) and each case pre-emergence growth was related to soil temperature, soil resistance, depth of burial and

seed weight. Vleeshouwers (1997) argued that the relative constancy of the defined relationships between species suggested that pre-emergence growth was governed by a common set of physiological principles. As such, it was further suggested that adaptation of the model to other weed species required only the identification of appropriate parameter values. However it was recognised that for field application of the model, further information regarding the vertical distribution of seeds within soil would be required and the model would have to be adapted to account for temperature variability over time and heterogeneity in soil compaction.

4.6. Model development requirements for G. aparine *and* S. media

It should be clear from this review, that the work of Bouwmeester & Karssen (1992, 1993a, b, c) and Vleeshouwers (1997) should be most influential in the development of our understanding of weed seedling emergence. This is because their models incorporate the greatest level of ecophysiological understanding. It should also be evident that the work of van der Weide (1993) on *G. aparine* that emergence is also of direct relevance. Given that Bouwmeester & Karssen (1992, 1993a, b, c) and Vleeshouwers (1997) provide our starting point in developing models of seed dormancy and germination for *S. media* and further developing such models for *G. aparine*, it is important to identify the main weaknesses of these models.

4.6.1. Weaknesses of preferred models of seed dormancy

- Restricted to summer annual weed species.
- Parameterised using only a single population.
- Vleeshouwers (1997) model of dormancy involves a complex switch mechanism for dormancy regulation. This introduces additional parameters and the consequent increase in model complexity that is difficult to justify given the lack of substantial evidence to support the existence of such a mechanism.

4.6.2. Weaknesses of preferred models for seed germination

- Parameterised using only a single population.
- Failure to account for effects of soil water potential in determining the extent and timing of seed germination.

- Developed using data from experiments conducted only at constant temperatures.
- Nitrogen is either present or absent.
- Light is either present or absent.
- Dormancy affects only the extent, not the rate of germination.

4.6.3. Weaknesses of model formulation

The models of Bouwmeester & Karssen (1992, 1993a, b, c) were formulated at the population level using differential equations and were entirely deterministic. This deterministic approach has a number of general problems as applied to the process of weed seed germination. Firstly, deterministic models do not explicitly account for the variation inherent within the process. Secondly, a related problem is that of model fitting and parameter estimation (see section 4.3.4).

In the model of Vleeshouwers (1997) dormancy cycling was formulated in continuous time using differential equations. However, stochasticity was introduced in an *ad hoc* way in that germination was modelled on an individual basis determined by a stochastic rule. This compared the time that active phytochrome (Pfr) was present with the time of dark reversion to inactive phytochrome (Pr) for a given temperature. Although the incorporation of stochasticity, albeit in this limited way countered some of the criticisms applied to Bouwmeester and Karssen (1992, 1993a, b, c), some problems related to model fitting and presentation remained.

Firstly, presentation of model output did not capitalise on the advantages of the stochastic formulation in that confidence intervals were not given for predicted means. In addition, estimation of model parameters was achieved by minimising the difference between experimental data and the model output, averaged over many runs. This did not allow confidence intervals to be attached to the ranges of parameters quoted from sensitivity analysis and a preferable method would be to use maximum likelihood estimation (Hunter, Glasbey and Naylor 1984). The main weaknesses of the models of Vleeshouwers (1997) and Bouwmeester & Karssen (1992, 1993a, b, c) are summarised below.

- Statistically questionable estimation of parameters and associated confidence intervals.
- No confidence intervals for model predictions.

The lists presented in sections 4.6.1, 4.6.2 and 4.6.3 show that there are several requirements to fulfil the modelling objective of this thesis. These include the identification of data for reformulation of existing models to winter annual species; reformulation of existing models to incorporate soil water effects and further model development to improve parameter estimation or fitting procedures and to allow models to account for observed variation. It should also be noted that in comparison with the models published by Vleeshouwers (1997), there is also potential to reduced model complexity in terms of the number of fitted parameters.

The identification of data for adaptation and reformulation of existing models requires a thorough review of published ecological data for *S. media* and *G. aparine*. This will be addressed in the next sections of this Chapter (Sections 4.6.4 & 4.6.5). This will also allow the identification of information needs (Section 4.7), which will then be targeted by new research within this thesis.

The need to incorporate soil water effects has already been described and this will be addressed in Chapter 11, following a detailed examination of hydrothermal time models as applied to *S. media* and *G. aparine* in Chapters 6 and 7. As such, the application of hydrothermal time models will not be further reviewed in this Chapter. Model development will be described in Chapter 11. The models to be developed will incorporate existing information with data gathered in this thesis, including that related to population differentiation. Details of the alternative techniques used to improve parameter estimates and associated confidence intervals will also be given in Chapter 11.

4.6.4. Existing information for seed dormancy

Existing information describing annual dormancy cycles in *S. media* is limited. However it has been shown that *S. media* forms a persistent seed bank, with seed longevity estimated to be greater than 20 years (Fitter & Peat, 1994) and therefore it is thought highly likely that *S. media* should have evolved some form of seed dormancy.

Baskin & Baskin (1976 & 1986) and Grime *et al.*, (1981) showed that new seeds of *S. media*, dispersed in the early summer were highly dormant. Baskin & Baskin (1986) recorded germination for freshly harvested *S. media* seeds and for the same seed lot, after four months burial at 7 cm in soil maintained at either 5 °C or a range of alternating temperatures. Their

work showed that high summer temperatures relieved dormancy, allowing seeds to germinate at a wide range of temperatures in the autumn. However they also noted that some dormancy relief also occurred at lower temperatures, though seeds could then only germinate at lower temperatures.

Whilst this information on initial dormancy relief is of interest, these papers did not assess changes in seed dormancy through an annual cycle of temperatures. Roberts & Lockett (1975) assessed the germination of a single population of freshly harvested *S. media* seeds and for the same seeds after approximately 4 weeks, 3 months and 9 months of either dry storage or seed burial. Seed germination was recorded at a range of constant and alternating temperatures either in distilled water or 20 mmol l^{-1} potassium nitrate solution with intermittent light, and this allowed some assessment of changes in seed dormancy. New seeds were again shown to be highly dormant and subsequent dormancy relief was shown to occur more rapidly for seed buried in moist field conditions compared to those stored dry at room temperature. Appreciable germination was recorded after 4 weeks, especially when tested in potassium nitrate solution, with alternating temperatures, but when tested again after 9 months, there were some indications that dormancy was increasing in the buried seeds, especially at high constant test temperatures. However, given that these seeds were tested at only infrequent intervals and given that no soil temperature data were presented, the usefulness of this data in describing seasonal changes in *S. media* dormancy is limited.

Similar problems relate to the observations of cyclic changes in dormancy in buried seeds of *S. media* recorded by Froud-Williams, Drennan & Chancellor (1984). Seed were again collected from a single population (albeit in two consecutive years), but again seeds were only recovered at infrequent intervals and no records of the soil temperature conditions were presented. Moreover the usefulness of this data is further limited by the fact that germination was only tested at 10/20 °C, and as such seasonal changes in the range of conditions allowing germination could not be assessed (Milberg & Andersson, 1998). This temperature restriction may also explain the anomalous result that recorded lower levels of dormancy in the summer in one batch of seeds. It is therefore clear that even with more substantive results, a model could not be formulated for seasonal changes in seed dormancy from the published data for *S. media.* Therefore in order to formulate a model of *S. media* dormancy and germination, burial experiments will need to be established and these will be presented in Chapter 5.

In contrast for *G. aparine* there are more comprehensive descriptions of both initial 'innate' dormancy and seasonal changes in seed dormancy (van der Weide 1993). Studies of seed persistence would suggest that dormancy is less important in the life history of *G. aparine* compared with *S. media*. This is because seed longevity has variously been estimated at less than 2 years (Brenchley & Warington, 1933), 2 years (Grime *et al.*, 1988) between 2 and 3 years (Holm *et al.*, 1997) and between 1 and 5 years (Fitter & Peat, 1994). Aarts (1986) estimated annual decay rates for seed buried at 20 cm of 0.63 with soil disturbance and 0.18 where the seed was left undisturbed.

Van der Weide (1993) reported that levels of initial 'innate' dormancy were variable and this is supported by contradictory reports of no dormancy by Froud-Williams, Chancellor & Drennan (1984) and Sjostedt (1959 in Malik & van den Born, 1988), compared with reports of high levels of initial dormancy in Grime *et al.* (1981). Additionally Froud-Williams (1985) noted variation in the level of initial 'innate' dormancy between field and hedge populations of *G. aparine*. Van der Weide (1993) showed that variation in the initial dormancy recorded in her experiments was related to harvest date and seed maturity, with early harvested and immature (green) seeds shown to be highly dormant. However it should be noted that experiments were restricted to a single set of conditions, namely 8 °C with 12.5 mmol l^{-1} potassium nitrate solution and this limits our interpretation of the actual extent of seed dormancy (Milberg & Andersson, 1998).

Van der Weide (1993) also showed that this initial 'innate' dormancy was relieved over time in dry storage, with the rate of dormancy relief higher at 20 °C in light (light conditions not described). Interestingly in another experiment, this time with mature seeds, in moist storage and with seeds tested over a range of temperatures, initial dormancy resulted in high levels of germination at low temperatures and low levels of germination at temperatures greater than 12 °C. Moist storage for two weeks at 5 °C relieved dormancy and seeds germinated to a high percentage across the temperature range from 5 to 20 °C. This contrasted with moist storage for 4 to 6 weeks at 15 or 20 °C, which induced dormancy such that germination at the lower temperatures was also significantly reduced. However, after 10 weeks at 15 or 20 °C there was evidence of change in dormancy. Increasing germination was recorded at the lower temperatures, though the maximum temperature for germination was still lower than that recorded initially.

Van der Weide (1993) conducted a year-long burial experiment with immature (green) seeds, buried in a field at 20 cm depth in compacted clay soil. The immaturity of seeds meant that initial 'innate' levels of dormancy were relatively high and seed germination was largely restricted to temperatures between 7 and 12 °C. Over the winter this temperature range widened, and high levels of germination were recorded in April between 7 and 17 °C, with germination also occurring at temperatures greater than 20 °C (but < 30 °C). In June, low levels of seed germination were recorded and germination was restricted to a narrower temperature range between 7 and 15 °C. However, by September, high levels of seed germination were again recorded across a temperature range between 5 and 20 °C. Van der Weide (1993) showed that these changes in seed dormancy when overlaid with soil temperature data, meant that significant seed germination occurred between mid-September and mid-November and between March and May. This gave good correspondence to the patterns of *G. aparine* emergence recorded in the field (Malik & van den Born, 1988; van der Weide, 1993). Moreover these data also matched the general pattern of dormancy cycling in winter annuals i.e. increasing dormancy relief in late summer and increasing dormancy induction in spring (Baskin & Baskin, 1986, 1995). Given the general credibility of these data, it was decided that efforts to advance our understanding of seasonal changes in seed dormancy for *G. aparine* were best focused on model development using the data presented by van der Weide (1993).

4.6.5. Existing information for seed germination

4.6.5.1. Water

In general there has been limited research into the effects of water availability on seed germination for both species.

Investigation of the effect of water availability on *S. media* germination has been limited to Grundy (1997) who, as previously discussed, combined the effect of water potential and temperature for a single population in a partial application of the hydrothermal model. This will not be discussed again in this section, as the hydrothermal model will be reviewed in detail in Chapter 6. As such it will suffice to note that Grundy (1997) showed that the final percentage of seeds germinated and the rate of seed germination both declined with decreasing water potential from 0 to -1.4 MPa.

For *G. aparine* observations on the effect of water availability on seed germination are limited to records by Hirinda (1959 in Malik & van den Born, 1988), Ferris (1988) and van der Weide (1993). Malik & van den Born (1987) also recorded significant reductions in final percentage germination of the closely related *G. spurium* at water potentials less -0.25 MPa, with no germination recorded below 0.75 MPa.

Hirinda (1959 in Malik & van den Born, 1988) recorded that seed germination was maximised at 40 to 60 % field capacity and declined above 80 %. It is not clear how this equates to equivalent soil water potentials. Ferris (1988) recorded significant reductions in *G. aparine* seedling emergence with alternate day compared to daily watering, especially in the field population (only one field and hedgerow population tested). In contrast van der Weide (1993) recorded no significant differences in *G. aparine* germination between a field and hedgerow population tested over a range of soil moisture contents (9 to 30 % moisture content). Given that these experiments were conducted in a clay soil, this may approximate to a range in soil water potentials from 0 to -1.5 MPa (Rundel & Jarrell, 1989). Van der Weide (1993) also showed a significant interaction between temperature and soil water content, with a significant and increasing reduction in seed germination recorded as soil water content decreased from 18 to 9 % (or -0.6 to -1.5 MPa (Rundel & Jarrell, 1989)) at 14 °C, but not at 8 °C. Interestingly van der Weide (1993) concluded on the basis of these data that *G. aparine* was insensitive to water availability. However is should be noted that these results concerned final germination percentages only and not the timing of germination which, as already discussed is often significantly effected by reduction in soil water availability (Roberts & Potter, 1980; Roberts, 1984). Moreover these data were also collected using either single populations or paired populations from contrasting local habitats and these were tested in only a limited set of conditions. As such, it should be clear that these results are insufficient to be incorporated in a model to describe the effect of water potential on *G. aparine* germination. The effects of temperature and water potential on *G. aparine* germination will be combined in a further investigation of the hydrothermal model in Chapter 7.

The effect of soil water content on seed production, seed size characteristics and subsequent germination patterns was investigated by Ferris (1988). These were compared for plants grown and seed produced in soils maintained at field capacity, 75 % and 50 % field capacity. Field capacity was defined with a soil water content of 20 % and therefore assuming that the soil was sandy loam in texture, field capacity corresponded to 0 MPa, 75 % field capacity to

-0.02 MPa and 50 % field capacity to –0.2 MPa (Rundel & Jarrell, 1989). Interestingly reduced soil water content significantly reduced plant size and decreased seed production, as measured by total seed weight and total seed number. The decrease in seed number with low soil water content was recorded despite an increase in the proportion of large seeds produced in the field capacity treatment. Subsequent seed germination was tested in a range of conditions (not including different water potentials) and some significant differences were recorded according to pre-treatments. It was however difficult to identify a general pattern in the levels of germination recorded according to previous soil water content.

4.6.5.2. Temperature

The effects of temperature on *S. media* germination have variously been described in Baskin & Baskin (1976 & 1986), Roberts & Lockett (1975), Thompson, Grime & Mason (1977), van der Vegte (1978), Grime *et al.* (1981), Thomson & Whatley, 1983, 1984 and Grundy (1997). It is clear that the results for final percentage germination are variable and depend largely on seed age and seed history. Roberts & Lockett (1975) and Baskin & Baskin (1976, 1986) considered the effects of seed age and storage conditions and van der Vegte (1978) and Grundy (1997) considered the effects of seed history and population differences on *S. media* germination in response to temperature.

The interaction of temperature with seed history and changes in seed dormancy was discussed in Section 4.6.4 and limitations in our understanding of the determinants of final percentage germination were identified. As such the interaction between seed dormancy and test temperature will not be further discussed. However it is worth noting that optimal temperatures can be defined, where seed germination is maximised within the constraints imposed by seed dormancy. These optimal temperatures for *S. media* have variously been defined as between 10 and 12 °C (Roberts & Lockett, 1975) and between 15 and 20 °C (Grundy, 1997), with the maximum temperature for germination defined at 30 °C (Roberts & Lockett, 1975) and 28 °C (Grime, Hodgson & Hunt, 1988) and the minimum defined at 5 °C (Grundy, 1997) and 10 °C (Grime et al. 1988).

Germination of buried *S. media* seeds was reported to be promoted by diurnal temperature fluctuations, both in light and darkness (Thompson & Whatley, 1984). The number of emerged seedlings was higher in the light and increased in both light and dark treatments with increasing mean amplitude of temperature variation from 4.8 to 14.4 °C and then

decreased as amplitudes further increased from 14.4 to 24 °C. These different amplitudes were achieved using a thermogradient bar, with one end maintained at a constant 12 °C and the other varying from 32 °C for 14 hours to 4.8 °C for 10 hours. This means that the temperature range varied with width of amplitude from alternating 11/15 °C for amplitudes of 4.8 °C, 9/23 °C for 14.4 °C and 5/30 °C for 24 °C. Correspondingly, Thompson & Whatley (1984) noted that these higher amplitudes were associated with temperatures that they considered outwith the limits for high germination at constant temperature (i.e. 30 °C). They also suggested that high amplitudes of temperature fluctuation tended to be associated with situations where insolation was high and soil moisture was low, that is, conditions that would not generally favour *S. media* germination. It should be noted that similar results were reported by Thompson & Grime (1983), albeit in this case the seed germinated in darkness showed a greater response to alternating temperatures than those germinated in the light, though high levels of variation in the results were noted.

The effects of temperature on *G. aparine* germination have been variously described by Froud-Williams (1985), Malik & van den Born (1988), Grime *et. al.* (1981) and van der Weide (1993). In common with *S. media*, the results for *G. aparine* show that the effects of temperature on final percentage germination are variable and depend on seed age and seed history, such that they are best understood in the context of known seed dormancy characteristics. However again optimal temperatures have been defined, ranging from 7 to 13 °C (Lauer, 1953 in Malik & van den Born, 1988), 12 to 15 °C (Sjostedt, 1959 in Malik & van den Born, 1988), 10 to 20 °C (Arai, 1961 in Malik & van den Born, 1988), 0.5 to 12 °C (Kurth, 1967 in Malik & van den Born, 1988) and 9 to 12 °C for field and 10 to 14 °C for hedgerow populations (Froud-Williams, 1985). There are fewer references to maximum and minimum temperatures and these have been defined as 20 °C and between 2 and 5 °C (Lauer, 1953 in Malik & van den Born, 1988), 26 and 6 °C (Grime, Hodgson & Hunt, 1988) and 15 and 5 °C for field and > 20 and < 5 °C for hedgerow populations respectively (Froud-Williams, 1985). Van der Weide (1993) noted that daily temperature fluctuations had no effect on the germination process, though no data to support this assertion were presented. This contrasted with the result given by Ferris (1988) who recorded significantly higher germination of *G. aparine* with alternating temperatures of 16/8 °C (8 hour day) compared with a constant 10 °C. These results were particularly significant for field populations of *G. aparine* and were conducted both with 8.8 μmol m^{-2} s^{-2} and without light.

Temperature also affects the rate of seed germination and for *S. media* this has been shown most clearly in Grundy (1997). The speed of germination was fastest at 20 °C and this temperature tended to maximise seed germination amongst the seed lots tested. Grundy (1997) extended the examination of germination rate and temperature to define a base temperature at 4.7 °C. This was used to calculate thermal time requirements for *S. media* germination. This is the only known example of a thermal time model applied to *S. media* germination time courses. This model was applied only to data from a single population. As such, further applications of this approach would be useful in order to quantify the extent of variation between populations.

Van der Weide (1993) explored the effect of temperature on germination rate for *G. aparine*. From data presented graphically, it appeared that germination rate was highest at 18 °C, though this was also the highest test temperature presented. The relationship between germination rate and temperature was used to define a base temperature of 2.5 °C and the thermal time requirement for seeds germinated on a thermogradient bar (details in Thompson & Grime, 1983) was defined as 75.2 degree days. This study again involved only a single population and it is clear that further investigations at higher temperatures would be useful.

4.6.5.3. Light

Investigations concerning the effect of light on *S. media* seed germination have given contradictory reports. *S. media* has variously been described as having no light requirement (Grime *et al.*, 1988), a partial light requirement (Baskin & Baskin, 1979; Baskin & Baskin, 1988, Kryger Jensen, 1995, Roberts & Lockett, 1975) and a complete light requirement for germination (Wesson & Wareing, 1969a, b, Froud-Williams, Drennan & Chancellor, 1984). These differences have been related to seed history age and storage conditions.

Grime *et al.* (1988) recorded no effect of light (40 W m^{-2} or 184 μmol m^{-2} s^{-1} following Hall *et al.*, 1993) on germination of *S. media* seeds stored dry in darkness at 5 °C for a maximum of six months and tested under alternating temperatures of 20/15 °C with 15 hours daylength. Roberts & Lockett (1975) reported similar results showing no effect of intermittent daylight on the germination of three year old dry stored seeds of *S. media,* when tested at alternating temperatures. However intermittent daylight was shown to promote germination in the same seeds when tested at constant temperatures. Roberts & Lockett (1975) showed that significant germination of freshly harvested seeds of *S. media* only occurred in 20 mmol l^{-1} potassium nitrate solution with intermittent daylight and alternating temperatures.

Germination of freshly harvested seeds was shown to be insensitive to light by Wesson & Wareing (1969b) for seeds tested at a constant 21 °C. Wesson & Wareing (1969b) went on to demonstrate that a complete light requirement for germination of *S. media* can be induced during seed burial and Baskin & Baskin (1979) showed that seed burial can result in germination promotion by very low levels of light, including that provided by a green safety lamp. For seed buried for either 16 or 18 months, germination was promoted by a PFD of between 3.7 and 5.7 μmol m^{-2} s^{-1} (Baskin & Baskin, 1979). Froud-Williams *et al.* (1984) also demonstrated promotion of *S. media* seed germination by red light compared to far red light. Interestingly, Kryger Jensen (1995) showed a partial light requirement for *S. media* germination following a short period of soil burial and higher numbers of emerged seedlings were recorded from seeds buried in light compared to darkness, at both 4 and 8 mm depth of soil. It is suggested that these depths of soil were insufficient to completely exclude light, especially if the soil was relatively sandy and uncompacted (Pons, 1992, Bliss & Smith, 1985). For this reason these results may differ from those reported for seeds buried at 50 mm by Wesson & Wareing (1969a) and for seeds sealed in black polythene by Froud-Williams *et al.* (1984).

Investigations concerning the effect of light on *G. aparine* seed germination have been less contradictory, though with a few exceptions (Froud-Williams, 1985). It is generally accepted that germination of *G. aparine* is inhibited by high light intensities (Bliss & Smith, 1985, Malik & van den Born, 1988), but some promotion of germination at low light intensities has been noted (Grime *et al.*, 1981, Bliss & Smith, 1985) though Grime *et al.* (1988) and Ferris (1988) recorded no significant difference in levels of *G. aparine* germination or emergence, between light and dark conditions.

Bliss & Smith (1985) attributed inhibition of *G. aparine* germination on the soil surface to photon flux densities greater than 70 μmol m^{-2} s^{-1} to and Sjostedt (1959 in Malik & van den Born, 1988) described light inhibition of *G. aparine* germination at 20 % of full daylight for freshly harvested and one year old seeds. Light inhibition of seed germination has been recorded for the closely related *Galium spurium* by Malik & van den Born (1987). However Froud-Williams (1985) recorded that light generally promoted *G. aparine* germination compared to that recorded in darkness, though inhibitory effects of light at low temperatures (alternating 10/4 °C) were recorded, though details of the specific light environment were not given.

Bliss & Smith (1985) reported light stimulation of *G. aparine* germination for seeds buried at 10 mm with a photon flux density estimated at only 0.026 μmol m^{-2} s^{-1}. Similarly Grime *et al.* (1981) showed that germination was promoted by photon flux densities of 4.5 µmol m^{-2} s^{-1} (converted from 0.97 W m^{-2} following Hall *et al.*, 1993) compared to germination in light (40 W m^{-2} or 184 µmol m^{-2} s^{-1} following Hall *et al.*, 1993) and complete darkness. Bliss & Smith (1985) suggested that germination stimulation at such low light intensities might indicate a lack of dark-reversion of Pfr, which would allow integration of light over longer periods, although this clearly requires further investigation. Pons (1992) also suggested that this could be the mechanism by which some seeds exhibit sensitivity to the low photon fluxes given by green safe light in dark treatments of germination experiments. However Grime *et al.* (1981) showed that *G. aparine* germination was not significantly promoted by short exposure to green safe lights at alternating temperatures.

Ferris (1988) recorded no overall differences between seedling emergence with light (8.8 µmol m^{-2} s^{-1}) or in darkness, though it should be noted that significant interactions between temperature and light conditions were also recorded. These meant that all except one of the six populations tested exhibited relatively higher emergence in darkness at constant (10 °C) compared to alternating temperatures (16/8 °C), though overall seedling emergence was higher with alternating temperatures. In another experiment with a paired field and hedge population, Ferris (1988) showed that average seedling emergence of the field population only was lower in full light compared to two shade treatments.

4.6.5.4. Nitrogen

Records for the effect of nitrogen on *S. media* seed germination are restricted to those published by Roberts & Lockett (1975). This is despite the widely reported preference of *S. media* for habitats with high levels of nitrogen supply (Sobey, 1981) and reported increases in *S. media* abundance with applications of both mineral fertiliser (Roberts, 1962) and organic manure (Roberts, 1962). Observations have shown that *S. media* is particularly sensitive to nitrogen concentration in water culture (Sinha, 1965 in Sobey 1981), and there is evidence that *S. media* accumulates high concentrations of nitrogen in plant tissues (Sobey, 1981). It has also been shown that *S. media* more effectively exploited top dressings of mineral nitrogen fertiliser compared with the cabbage crop plants and a number of other weed species (Lawson & Wiseman, 1979).

Roberts & Lockett (1975) showed that freshly harvested *S. media* seeds failed to germinate in water, but low levels of seed germination were recorded for seeds placed in 20 mmol l^{-1} potassium nitrate solution. Similar results were found in experiments using dry stored or buried seeds of *S. media*, with germination in intermittent light substantially promoted by 20 mmol l^{-1} potassium nitrate solution, especially with alternating temperatures.

The wider effects of nitrogen, in different formulations and concentrations, on seed germination for *S. media* have been inadequately investigated, especially when considered with respect to the reported importance of nitrogen in other parts of the plant life cycle. Chapter 10 will investigate the effect of different nitrogen ions and concentrations on *S. media* germination, together with the effect of soil nitrogen on seed production and subsequent patterns of seed germination.

In contrast, more data are available describing the effect of nitrogen on *G. aparine* germination. Sjostedt (1959 in Malik & van den Born, 1988) reported that *G. aparine* germinated to a higher level in soil and soil extracts compared to distilled water. This was attributed to stimulation by soil nitrates. Froud-Williams (1985) reported that seed germination of field populations (but not hedgerow populations) was promoted in 200 mmol l^{-1} potassium nitrate solution. This was further investigated by Ferris (1988) who reported *G. aparine* germination for three paired field and hedgerow populations in water, 2, 20 and 200 mmol l^{-1} potassium nitrate solutions. Although no clear dose response was recorded, germination of the field populations was significantly higher in the 2 and 20 mmol l^{-1} potassium nitrate solutions compared with the distilled water control. Germination of all populations was significantly reduced in 200 mmol l^{-1} potassium nitrate suggesting that the 200 mmol l^{-1} potassium nitrate was supra-optimal. This corresponds with observations for other plants as detailed in Chapter 1 (Karssen & Hilhorst, 1992). Van der Weide (1993) showed that seed from arable populations had an absolute nitrogen requirement and that germination was optimal at potassium nitrate concentrations between 6.25 and 25 mmol l^{-1}.

In a field experiment, Ferris (1988) investigated the effect of soil nitrogen, supplied at either 0, 100 or 200 kg N ha^{-1} on plant morphology and seed production. Seed size characteristics were assessed and subsequent germination patterns examined in controlled environment experiments. The supply of additional soil nitrogen significantly reduced seed production (as measured by seed fresh weight). There were no significant differences in the number of

seeds produced, plant fresh and dry weights or the plant to seed weight ratio, according to nitrogen treatments. The difference in total seed fresh weight is likely to be a result of an increased proportion of larger seeds in the low or no nitrogen treatments. Subsequent seed germination was tested in a range of conditions and overall germination of the seeds produced following application of 100 kg N ha^{-1} was significantly reduced compared with 0 or 200 kg N ha^{-1} treatments.

It is clear from sections 4.6.4 and 4.6.5 that the two selected species differ in their fundamental biology and in the extent to which data describing the effect of different environmental factors on seed dormancy and germination is described. As such, the information needs for model development for *S. media* and *G. aparine* are summarised below.

4.7. Summary of information requirements for model development

Table 4.1 summarises the information required for the development of quantitative models of seed dormancy and germination for *S. media* and *G. aparine*. It also identifies where there is existing data and lists the chapters that address each data inadequacy. This table has been compiled from review of existing information, as detailed in sections 4.6.4 and 4.6.5 and from recognition of general issues in developing models of weed seed dormancy and germination (section 4.5).

Table 4.1 shows that there is particular emphasis on quantifying the extent of variation, between distinct populations and related to the environment during seed production. For *G. aparine*, data gathering was restricted to this quantitative assessment of variation and assessment of the combined effect of temperature and water potential on seed germination. For *S. media*, data requirements were broader and issues of variation were addressed together with seasonal variation in seed dormancy, and the effects of temperature, water potential, light and nitrate on seed germination. For both species, use of contrasting populations in assessment of the combined effects of temperature and water potential on seed germination allowed a robust test of the utility of the hydrothermal model for seed germination (see section 4.5.2).

Information requirements	*Stellaria media*	*Galium aparine*
• Seasonal changes in seed dormancy	Chapter 5	van der Weide (1993)
• Combined effect of temperature and water potential on seed germination	Chapter 6 Grundy (1997)	Chapter 7
• Effect of light on seed germination	Chapter 6	Bliss & Smith (1985) Grime *et al.* (1981)
• Effect of nitrate on seed germination	Chapter 10	Ferris (1988) van der Weide (1993)
• Effect of genetic variation between populations on initial seed dormancy and germination	Chapters 2, 5, 6, 8 & 10	Chapters 3, 7 & 9
• Effect of environmental variation during seed production on seed dormancy and germination	Chapters 8 & 10	Chapter 9
• Interaction between genetic and environmental variation on seed dormancy and germination	Chapters 8 & 10	Chapter 9

Table 4.1: Summary of information requirements for developing quantitative models of seed dormancy and germination for *Stellaria media* and *Galium aparine*.

Chapter 5. Dormancy cycling in *Stellaria media*

5.1. Summary

A seed burial experiment was conducted with *Stellaria media* seed from three contrasting populations. Seeds were buried at 5 cm and at 10 cm in an agricultural soil and samples were exhumed monthly to assess seasonal changes in seed dormancy. Daily minimum and maximum soil temperatures at 10 cm were recorded.

Initial dormancy varied between populations. Where seed dormancy was low, high levels of fatal seed germination were recorded, particularly for the Perthshire population. It was suspected that this population also lacked a light requirement for germination.

Seasonal changes in dormancy were evident in germination tests with exhumed seeds. Detection of these changes depended on germination assessment at a range of temperatures. The general pattern of seasonal change in dormancy was common to all three populations. Dormancy release in the autumn widened the range of temperatures at which seeds germinated, initially restricting germination to temperatures less than 30 °C. Dormancy induction in the spring narrowed the range of temperatures at which seeds germinated, increasingly restricting germination to temperatures less than 30 °C. Patterns of dormancy release in the second autumn were ill determined, largely through lack of extended time-series data.

Significant reductions in seed viability were recorded in two instances of suspected soil borne fungal infection. Seeds from all three populations were affected. This demonstrated the importance of replication in the design of seed burial experiments. More importantly it also suggested an option for developing biological control of *S. media* seeds.

5.2. Introduction

Dormancy is a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate (Vleeshouwers, 1997).

Newly dispersed seeds are often highly dormant and fail to germinate in conditions that subsequently favour germination. This 'innate' dormancy has been demonstrated in a wide range of weed species, including *Stellaria media* (Baskin & Baskin, 1976; Baskin & Baskin, 1986). Seeds of *S. media* and other winter annual species dispersed in spring or early summer often have high levels of initial dormancy. This is thought to have evolved to prevent germination during short periods of wet weather, in the otherwise hot and dry summer months. Baskin & Baskin (1986) showed that high summer temperatures relieved this dormancy, increasing the proportion of seeds able to germinate in the prevailing conditions of a cool and wet autumn. In contrast, for many summer annual species, low winter temperatures relieve dormancy leading to a high proportion of seeds germinating in the spring after dispersal (Baskin & Baskin, 1987).

Whilst 'innate' dormancy has been shown to restrict seed germination to suitable conditions immediately after dispersal, seeds of many weed species are known to persist for long periods in the soil (Mortimer, 1990) and to exhibit characteristic seasonal patterns of seedling emergence (Roberts, 1964; Chancellor, 1986). These phenomena cannot be explained by temporary 'innate' dormancy, but instead by temporal patterns of changing seed dormancy.

Weed seed persistence in soil has been shown to result from the development of altered requirements for germination (Egley, 1995). Most commonly seed burial has been shown to result in development of a light requirement for seed germination (Wesson & Wareing, 1969b; Baskin & Baskin, 1979; Froud-Williams, Drennan & Chancellor, 1984). This has been used to explain the persistence of viable seeds of many weed species for many years in uncultivated soils (Murdoch & Ellis, 1992) and experiments with soil cultivation in the dark have had some success in reducing weed seed emergence (Scopel, Ballare & Radosevich, 1994; Kryger Jensen, 1995).

In many weed species, seasonal patterns of seedling emergence have been related to annual cycles of dormancy, i.e. periods of alternate dormancy induction and relief, resulting in gradual transitions between high and low dormancy (Egley, 1995). Dormancy induction results in a narrowing of the conditions that permit germination, whilst dormancy relief broadens the range of conditions in which seeds can germinate (Vleeshouwers, Bouwmeester & Karssen, 1995). To detect such dormancy cycles requires that seeds are buried and retrieved at regular intervals for germination testing under a range of conditions.

Seasonal loss and induction of dormancy in buried weed seeds was first reported for *Polygonum aviculare* (Courtney, 1968). Subsequently seasonal dormancy cycles have been illustrated in a large number of annual species, including *Aphanes arvensis* (Roberts & Neilson, 1980); *Lamium amplexicaule* and *L. purpureum* (Baskin *et al.*, 1986); *Polygonum persicaria* (Bouwmeester & Karssen, 1992); *Chenopodium album* (Bouwmeester & Karssen, 1993a); *Sisymbrium officinale* (Bouwmeester & Karssen, 1993b); *Spergula arvensis* (Bouwmeester & Karssen, 1993c) and *Viola arvensis* (Baskin & Baskin, 1995). Annual cycles of dormancy for *Galium aparine* were described in van der Weide (1993) and some observations of cyclic changes in dormancy were recorded for *S. media* in Roberts & Lockett (1975) and Froud-Williams, Drennan & Chancellor (1984).

As seedling emergence has been shown to relate to dormancy cycles and seasonal changes in the conditions which favour germination, temperature has been identified as the main influence on dormancy relief and induction (Bouwmeester & Karssen, 1989). Temperature varies over the same time scales as seed dormancy, unlike more instantaneous or unpredictable changes in soil water potential, soil nitrate concentrations and the soil light environment. Attempts to link patterns of seasonal changes in seed dormancy with environmental factors other than soil temperature have proved largely unsuccessful (Bouwmeester & Karssen, 1992, 1993a, b, c). It should be noted that soil temperatures are less variable than corresponding air temperatures as the soil matrix and solution buffer changes, with the insulating effect of soil increasing with depth. Egley (1995) noted that because the magnitude of diurnal and seasonal temperature differences decreases with increasing soil depth, there is argument that the effects of temperature on dormancy cycling should be reduced for seeds buried deep in the soil profile. However, there is little evidence to support this assertion.

Temperature has several roles in seedling emergence. In addition to being largely recognised as the main influence on changes in seed dormancy, temperature is also an important in determining the extent of seed germination and the rate of seed germination and emergence. Temperature is also related to the rate of seed deterioration in buried seeds (Rees, 1997) and these various reactions may complicate the temperature relationship with seedling emergence.

Seasonal cycles of dormancy in buried seeds affect the number of weed seedlings emerging following soil cultivation (Bouwmeester & Karssen, 1992, 1993a, b, c). Chapters 1 and 4 identified the importance of better prediction of weed seed germination in allowing more targeted weed control and it is clear that this relies on better understanding of the processes that determine seed dormancy.

As previously mentioned (section 4.6.4), van der Weide (1993) described seasonal variation in seed dormancy for *G. aparine* with germination requirements and soil temperatures coinciding between mid-September and mid-November and between March and May. Van der Weide (1993) also showed that the range of temperatures at which seeds were able to germinate was widest overwinter (3 - 20 °C) and most restricted in June and July (10 – 12 °C). Given that these data fit with the general model of dormancy cycling in winter annuals i.e. increasing dormancy relief in late summer and increasing dormancy induction in spring (Baskin & Baskin, 1986, 1995) and given that *G. aparine* seeds tend not to persist in the soil seedbank for more than five years (Grime, Hodgson & Hunt, 1988), it was decided to concentrate efforts on *S. media* dormancy.

S. media forms a persistent seed bank, with seed longevity variously reported to be greater than 20 years (Fitter & Peat, 1994) to between 40 and 60 years (Salisbury, 1961 and Evans, 1962 respectively in Sobey, 1981). Annual declines in seed viability for seed buried undisturbed in soil have variously been reported as 19 % (Roberts & Feast, 1973) and 30 % (Roberts & Dawkins, 1967 in Sobey, 1981), although these were for seeds at depths of 15 and 23 cm respectively. Given that *S. media* has a persistent seed bank (Thompson, Bakker & Bekker, 1997), it is thought likely that it has evolved some form of seed dormancy.

As described earlier, *S. media* seeds often have 'innate' dormancy which precludes germination immediately after dispersal (Baskin & Baskin, 1976, 1986), but evidence for dormancy cycling is inconclusive. Froud-Williams, Drennan & Chancellor (1984) buried seeds from one population of *S. media* collected from the field in two consecutive years (one freshly harvested and one following a year of dry storage). Seeds were buried at 15 cm in July and germination was tested at 10/20 °C in diffuse light after 3, 6, 12 and 15 months of burial. Low levels of germination were recorded regardless of length of burial for the seeds previously stored dry for a year and germination of the freshly harvested population was higher in the following summer than in the spring or autumn. This was a curious result and not one that matches the patterns of *S. media* emergence that have been recorded in the field

(Roberts, 1964; Chancellor, 1986, Mortimer, 1990). However, germination was only tested with one set of alternating temperatures (10/20 °C) and the detection of seed dormancy cycles depends on the conditions under which germination experiments are conducted (Milberg & Andersson, 1998). Moreover there did not appear to have been any replication of the cylinders in which the seeds were buried and no records of soil temperature conditions during the study were presented by which it could be assessed whether conditions were typical in that year. Finally, as is a common theme with this thesis, the results were for one population only and it is therefore possible that this population itself was atypical.

It is a general criticism of studies of seed dormancy cycles that they have focused on a single population and made no attempt to assess variability in population behaviour or as a result of differences in maternal environment. Regarding intraspecific variation, the only exception was a study of four different populations of the winter annual *Emex australis* (Polygonaceae) for seeds placed on the soil surface and tested at monthly intervals at 15/25 °C (Panetta & Randall, 1993). Seed germination was shown to vary seasonally, but although the general patterns were consistent between populations, populations differed markedly with respect to germination percentages especially between spring and summer. Regarding variation related to the maternal environment, Baskin & Baskin (1995) showed pronounced variation in the annual dormancy cycles of buried seeds from the same population of the winter annual, *Viola arvensis*, collected in different years and different months of the same year.

It is clear that existing information supporting or disproving the existence of annual dormancy cycles in *S. media* is inconclusive, although there is good reason to suspect that such cycling occurs. As such, one aim of this chapter is to identify whether or not *S. media* seeds vary seasonally in their germination requirements. Secondly in examining this question using three populations of the same species, it is hoped that the robustness of any patterns observed can be assessed. The issue of maternal effects on seasonal changes in *S. media* seed dormancy will not be addressed.

5.3. Methods

5.3.1. Seed source

For this experiment, additional seed was produced from the original seed of the selected populations collected in 1995 (described in section 2.3.1). The original seed had previously been stored dry in an incubator maintained at 10 ± 2 °C.

In November 1997, 30 pots (25.4 cm diameter) were filled with a standard peat and perlite mixture (Appendix 1). 10 pots were allocated to each population and labelled accordingly. 20 seeds of *S. media* from the named population were surface sown into each pot and the pots were placed in a completely randomised block in an unheated greenhouse. Pots were watered regularly and on emergence, seedlings were thinned to five plants per pot. On first flowering, whole pots were covered by a layer of muslin and sealed at the bottom with an elastic band, to prevent cross-pollination between populations.

Seeds were hand-harvested in June, September and October 1998, but only the seeds from September and October 1998 were used in this experiment. Seeds were bulked by population for each harvest data and immediately hand cleaned (using graded sieves). Cleaned seeds were stored in labelled trays lined with absorbent paper for five days prior to either germination testing or burial.

5.3.2. Seed burial

Prior to seed burial, seeds from each population were sorted into 24 samples. The September samples from the Leicestershire and Perthshire populations weighed 1 g and samples for the Caithness population weighed 2 g. The October samples from the Leicestershire and Perthshire populations weighed 0.4 g and samples from the Caithness population weighed 0.8 g. Differences in sample weights between populations reflected differences in seed size and between burial dates reflected differences in seed availability. This meant that assuming an average seed weight of 0.4 mg for the Leicestershire and Perthshire populations and 0.8 mg for the Caithness population, 2500 seeds per sample were buried in September and 1000 in October. Each sample was sown into a separate fine-mesh nylon bag (approximately 6 x 6 cm) and the bags were labelled by population codes, internally (with colour beads) and externally by permanent marker (population name).

On September 11 1998, 24 clay pots (22.9 cm diameter; single drainage hole) were buried in two blocks (each with two rows of six pots) along the northern edge of Anchordales Field, Boghall Farm (Ordnance Survey: Grid reference NT 250 655), Midlothian, Scotland. The pots were buried with 3 cm proud of the soil surface for ease of later removal and then filled with field soil to a uniform 5 cm short of the soil surface. A single bag of seeds from each population was placed in the pot and immediately covered with 5 cm of soil, such that light was prevented from reaching the seeds and the soil surface was continuous with that of the field. Initially, the depth of 5 cm was considered appropriate because emergence of seedlings of *S. media* from depths greater than 3 cm is minimal (Chancellor, 1964; Mead, Grundy & Bond, 1996). Following seed burial, cages, designed to prevent damage by small mammals, were then placed over the two blocks and the soil surface was kept clear of weeds and litter.

Following high levels of germination in seed samples from the Perthshire and Caithness populations buried at 5 cm in September 1998 (see section 5.4.1), additional seed samples were prepared and buried at 10 cm on 23 October 1998. It was decided to keep the samples buried at 5 cm for comparison and as it was not possible to set up a second series of experiments for logistical reasons, the new seed samples were buried at 10 cm in the same pots. In order to minimise the impact of unearthing the old seeds, the new seed samples were buried in darkness, between 23:00 and 01:00, using only a green safe light, provided by a battery powered torch filtered by with two layers of dark yellow-green Cinemoid filter (Lee filters #90).

Following seed burial, two randomly selected pots (one from each block) were unearthed each month. Pots were carefully uplifted, labelled with block number and sealed in black plastic bags for transport to the dark room and establishment of germination tests.

5.3.3. Seed germination

The germination characteristics of freshly harvested seeds and of buried seeds at monthly intervals from September 1998 were assessed at four constant temperatures (5, 10, 20 and 30 °C) following an initial exposure to red light (and subsequent intermittent exposure to very low levels of green light). Temperatures of 30 °C were higher than those typically recorded in the field (Figure 5.5).

Germination tests for freshly harvested seeds were started within a week of seed collection. For buried seeds, seeds were removed from the pots, surrounding soil and nylon bags in the dark room under a green safe light (described below). Where a proportion of the buried seeds had germinated, efforts were made to restrict the germination test to viable seeds only. These were selected by adding water to the sample of recovered seeds and selecting only those seeds that sank and that were full and firm (as determined by gently squeezing with tweezers).

Generally, for each germination test, 50 seeds were placed on a double layer of Whatman no. 181 paper in a 9 cm plastic Petri-dish. However it should be noted that for the Perthshire population, high levels of germination were recorded in the buried seeds. This restricted the number of seeds that could be recovered and therefore the number of seeds per germination test was less than 50 and equivalent to the maximum number of seeds recovered, as divided equally between replicates. There were four replicates for each population x temperature (two from each block of buried seeds). Each Petri dish received 10 ml of distilled water under a green safe light. The safe light was constructed by covering an Osram L 36W/30 fluorescent tube and diffuser with two layers of dark yellow-green Cinemoid filter (Lee filters #90), giving a photosynthetic photon flux of 0.51 ± 0.19 μmol m^{-2} s^{-1}. Petri-dishes were then sealed in a self-seal clear polythene bags to minimise water loss by evaporation and exposed to red light for 15 minutes. The red light was given by an Osram L 36W/30 fluorescent tube and diffuser covered with two layers of bright red Cinemoid filter (Lee filters #26), giving a photosynthetic photon flux of 3.84 ± 0.71 μmol m^{-2} s^{-1} (photosynthetic photon fluxes recorded by quantum sensor and a Campbell CR10 datalogger). The Petri dishes were randomly placed in sealed cardboard boxes wrapped in black polythene and lined with bubble wrap, with a separate box for each temperature using the green safe light. The boxes were then removed to unlit incubators (Gallenkamp/Cryotechnics cooled) maintained at the defined constant temperatures ($\pm$ 2 °C). Germination was assessed under the green safe light after 24, 36 and 48 hours, then daily for the next five days and then at less frequent intervals until germination ceased. Seed germination was defined as radicle emergence to greater than 1 mm and germinated seeds were removed from Petri dishes when recorded.

5.3.4. Seed viability

At the end of the germination test, the numbers of remaining seeds were counted. The remaining seeds were classified as 'alive' or 'dead', as determined by whether the seeds were full and firm (alive) or empty and/or soft (dead). This was assessed by gently squeezing the remaining seeds with tweezers.

5.3.5. Statistical analysis

For statistical analysis, final percentage germination was calculated as a function of the number of viable seeds in the germination test for each Petri-dish. This was plotted against time to determine whether seed dormancy decreased initially from seed harvest through the autumn and winter and whether seed dormancy subsequently increased over the summer months. Similarly the percentage of seeds that remained viable at the end of the experiment was calculated as a function of the number of seeds in the germination test and this was plotted against time to determine any changes in seed viability with increasing length of seed burial.

Changes in seed dormancy and seed viability were compared with soil temperature at 10 cm for the same period. Daily minimum and maximum soil temperatures at 10 cm (09:00) were kindly given by Colin McEvoy of the Forest Commission, Northern Research Station. Soil temperatures were recorded for a similar brown earth soil at the Northern Research Station on the Bush Estate (Ordnance Survey: Grid reference NT 249 640). This site was approximately 1.5 km from the field where the seeds were buried and difference in altitudes was no greater than 10 m.

5.4. Results

5.4.1. Fatal seed germination

As described in section 5.3.2, it was initially considered that seed burial at 5 cm depth in soil would be sufficient to limit seed germination. However it was immediately clear when seeds were first recovered a month after the September burial, that large numbers of seeds from the Perthshire and Caithness populations had germinated. Fatal seed germination was estimated by counting the proportion of empty seeds in 4 random samples of 50 seeds from each population. This showed fatal germination of 81 ± 3, 57 ± 2 and 17 ± 1 % of the buried

seeds for the Perthshire, Caithness and Leicestershire populations respectively. For this reason, additional seeds were buried in October at 10 cm (as described in section 5.3.2).

However it was again immediately clear that when seeds were recovered a month after the October burial at 10 cm, there had been substantial germination of seeds from the Perthshire and Caithness populations. This fatal seed germination was again estimated by counting the proportion of empty seeds in 4 random samples of 50 seeds for each population and this showed fatal germination of 89 ± 3, 54 ± 3 and 15 ± 2 % of the buried seeds for the Perthshire, Caithness and Leicestershire populations respectively. Given that sufficient seed could be recovered from the Caithness and Leicestershire populations for seed germination testing as planned, it was decided to proceed and to include investigations of the Perthshire population, albeit with a reduced size seed sample (see section 5.3.3)

5.4.2. Seed germination before and after burial

Before burial, initial seed germination varied between populations from 36 % for the Leicestershire population, to 85 % for Perthshire and 75 % for Caithness (at 10 °C). As such, populations varied in their initial dormancy.

Figure 5.1 and Figure 5.2 show the patterns of seasonal variation in germination of viable exhumed seeds of the three populations under different test conditions. The results are presented according to block (each with two replicates) because of anomalies between blocks. This was most apparent in April 1999 and was associated with a reduction in the proportion of viable seeds recovered from block 1 (Figure 5.3). This will be discussed in section 5.5 and further analysis will disregard results from block 1.

During the year-long burial experiment, seasonal changes in dormancy were evident in germination tests with exhumed seeds (Figure 5.2). Expression of these changes showed some dependence on temperature in the germination test, especially at 30 °C. The general pattern of seasonal change in dormancy was common to all three populations, with release of initial dormancy over winter and rapid dormancy induction in early summer. Limited germination was recorded at 30 °C throughout the year for the Leicestershire population. Germination tests at 30 °C for both the Perthshire and Caithness populations revealed gradual dormancy induction, with seed germination in the spring and early summer increasingly limited at higher test temperatures. This was particularly marked in the

Perthshire population, with dormancy induction apparent at 30 °C from late February onwards.

Figure 5.5 shows seasonal variations in daily minimum and maximum soil temperatures at 10 cm. Initial soil temperatures at burial were approximately 10 °C. The lowest temperature was 0.9 °C recorded in February 1999 and the highest temperature was 17.9 °C recorded in July 1999. July was the warmest month.

Figure 5.2 shows seasonal changes in germination of exhumed seeds from block 2, overlaid with daily minimum soil temperatures. From this it is clear that dormancy release was associated with decreasing soil temperatures in the autumn and dormancy induction was associated with increasing soil temperatures in the spring. It was also apparent that the rate of dormancy release in the autumn was relatively slow compared to dormancy induction in the spring.

There was marked variation between populations for patterns of dormancy release in August and September 1999, with high levels of viable seed germination recorded at 5, 10 & 20 °C for the Leicestershire population and steady increases at all temperatures for the Perthshire population. Germination of the Caithness population was limited and only marginally increased from levels recorded in July, 1999. However in October 1999 low levels of germination were recorded for all populations at all test temperatures. For the Caithness population this reflected a small increase in the proportion of seeds germinating from the low levels recorded in September 1999, but for the Leicestershire and Perthshire population this represented a change from low to high levels of dormancy. This was particularly marked for the Leicestershire population. This increase in seed dormancy did not appear to be associated with atypical soil temperature measurements in September or October 1999, or with marked changes in seed viability. It was noted that seed viability decreased markedly in September 1999, especially in the Caithness population. In contrast, the proportion of viable seeds recovered in October 1999 was high, but germination levels were low.

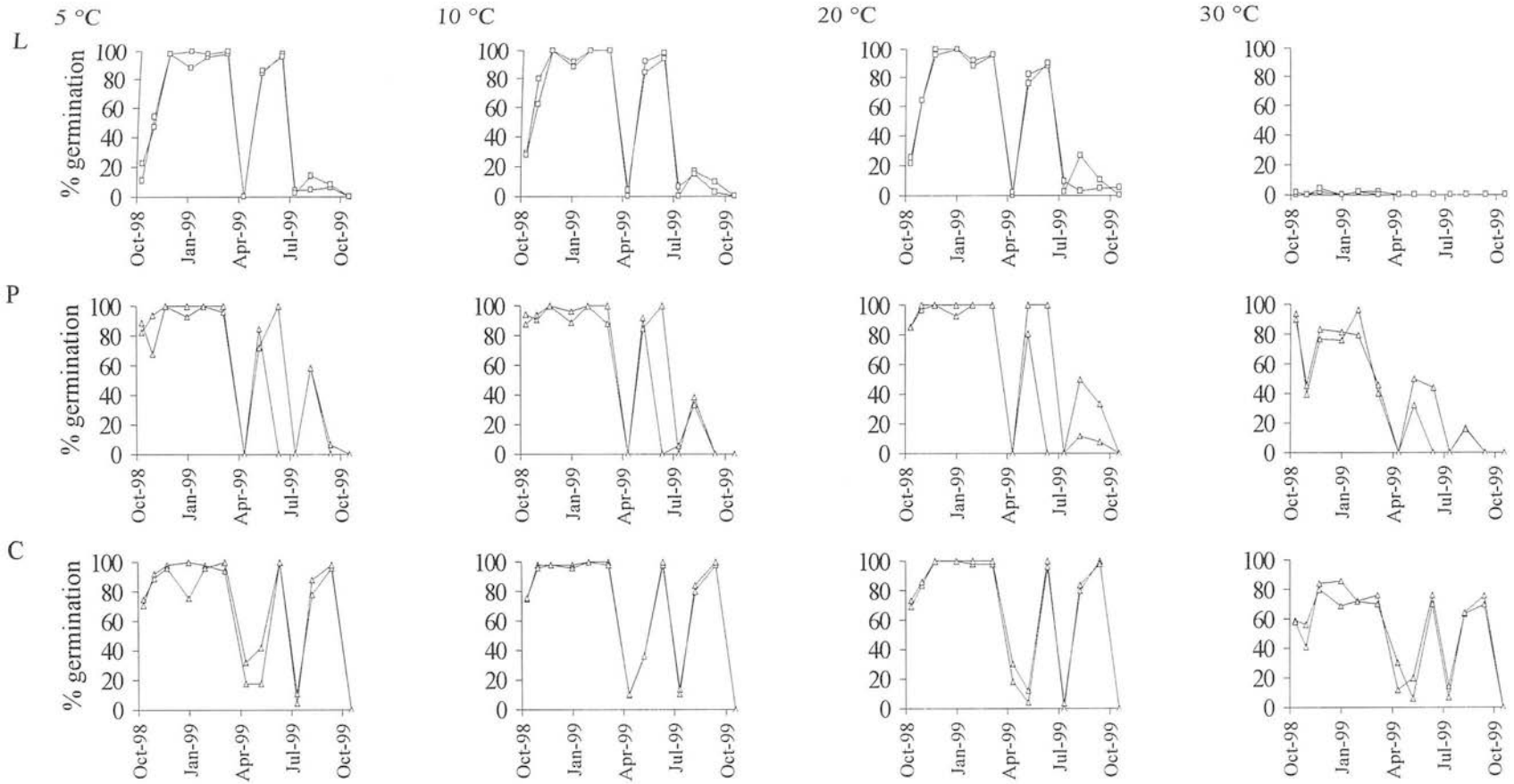


Figure 5.1: Seasonal variation in germination of viable exhumed seeds of *Stellaria media* populations under different temperature conditions: Block 1
Population codes: L: Leicestershire; P: Perthshire; C: Caithness. n = 2.

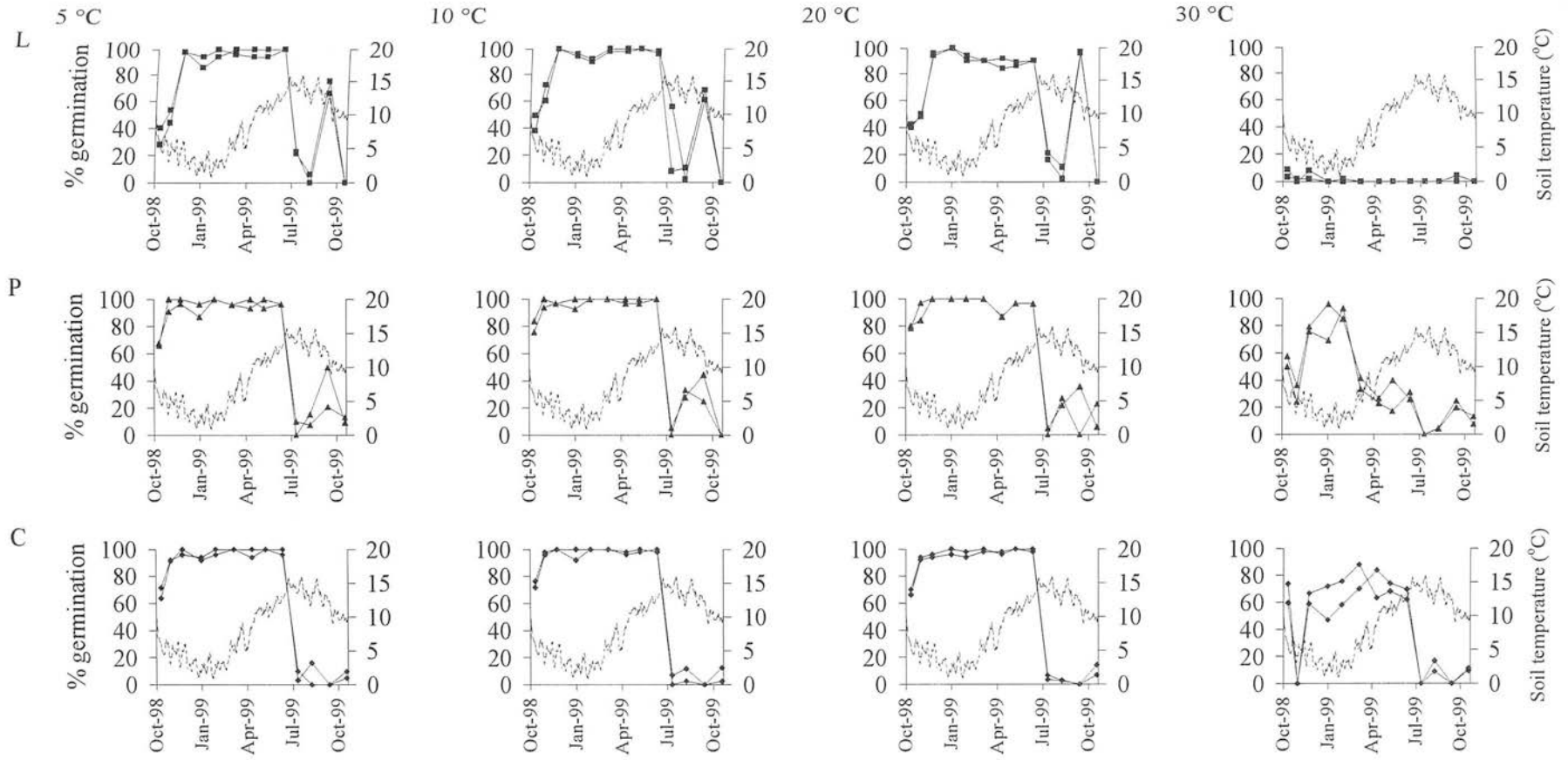


Figure 5.2: Seasonal variation in germination of viable exhumed seeds of *Stellaria media* populations under different temperature conditions: Block 2 with superimposed daily minimum soil temperatures.
Population codes: L: Leicestershire; P: Perthshire; C: Caithness. n = 2.

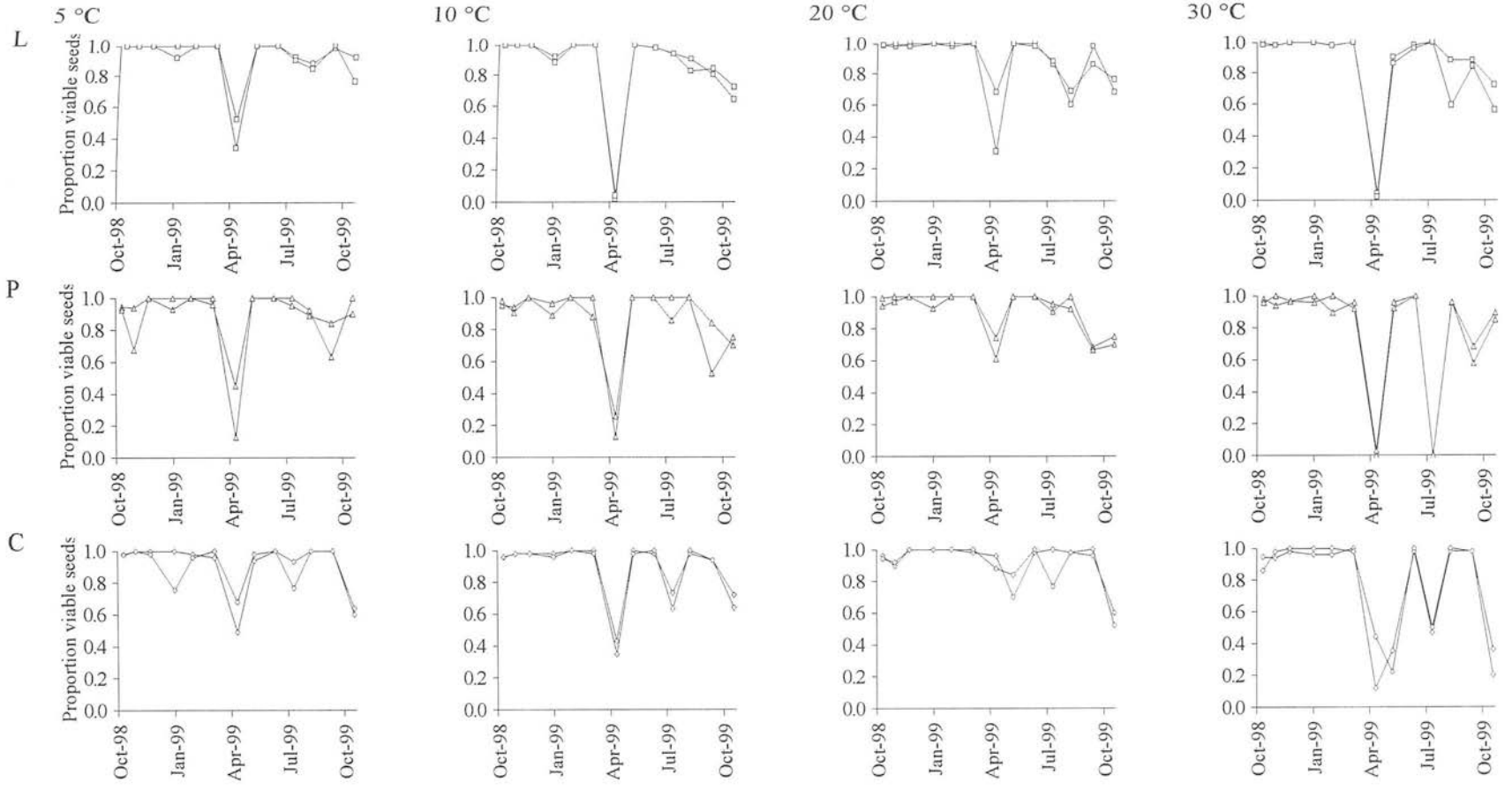


Figure 5.3: Seasonal variation in proportion of viable exhumed seeds of *Stellaria media* populations under different temperature conditions: Block 1
Population codes: L: Leicestershire; P: Perthshire; C: Caithness. n = 2.

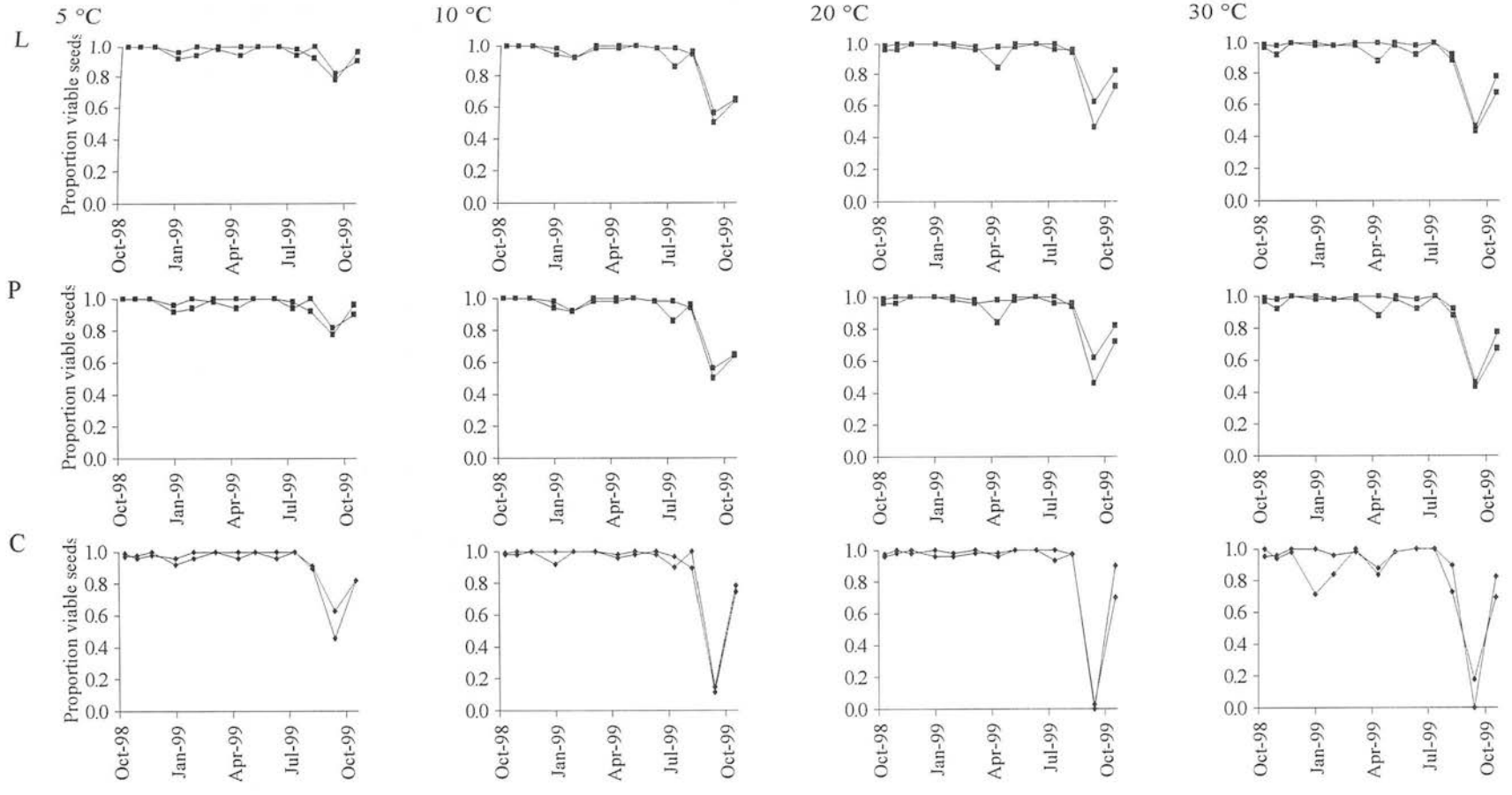


Figure 5.4: Seasonal variation in proportion of viable exhumed seeds of *Stellaria media* populations under different temperature conditions: Block 2
Population codes: L: Leicestershire; P: Perthshire; C: Caithness. n = 2.

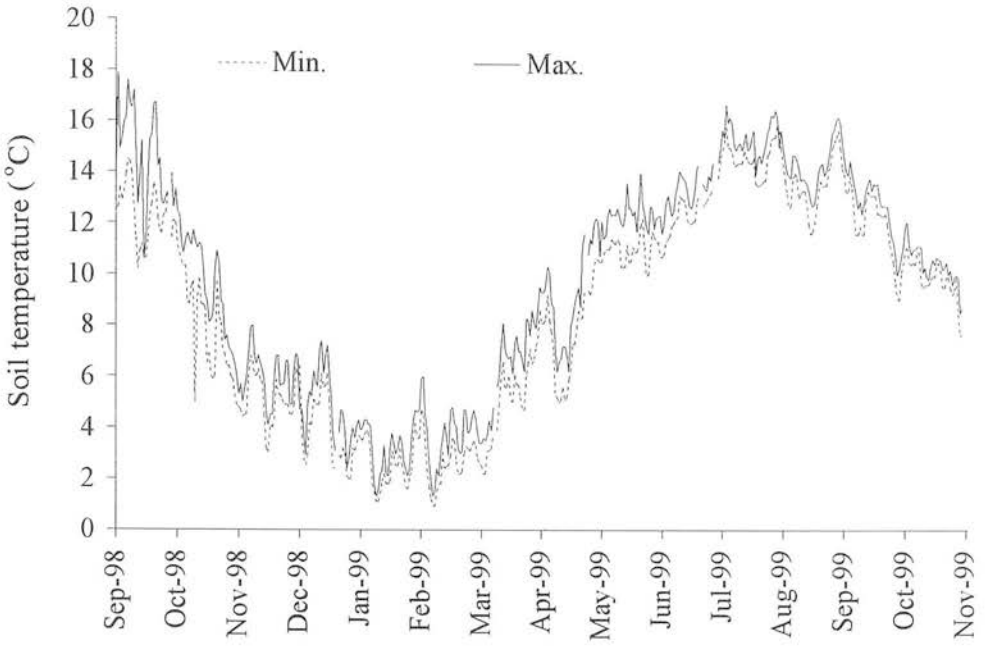


Figure 5.5: Daily minimum and maximum soil temperatures at 10 cm from Northern Research Station, Bush Estate 1998/99

5.4.3. Changes in the proportion of viable seeds

Seasonal changes were also evident in the proportion of viable seeds recorded at the end of each germination test with exhumed seeds (Figure 5.3 & Figure 5.4). These changes varied between blocks and between populations, with some dependence on temperature in the germination test. Generally a decreasing proportion of viable seeds were recovered from August 1999 onwards. Significant reductions in seed viability were recorded in two instances of suspected soil borne fungal infection, in April for block 1 and September for block 2. Seeds from all three populations were affected.

5.5. Discussion

Fatal seed germination occurs when seeds germinate, but fail to emerge as seedlings. Given that *S. media* seedling emergence from soil depths greater than 3 cm is minimal (Chancellor, 1964; Mead, Grundy & Bond, 1996), fatal seed germination was recorded when seeds germinated at both 5 and 10 cm depth in soil. The evolution of dormancy and light

requirements for germination is strongly linked with selective advantages associated with avoiding fatal seed germination (Mortimer, 1990).

5.5.1. Initial dormancy, germination requirements and fatal seed germination

The high levels of fatal germination recorded were surprising as newly produced seeds of *S. media* have been shown to be highly dormant (Baskin & Baskin, 1976, 1986; Grime *et al.*, 1981). However these results were for seeds harvested in the early summer, in contrast to the late summer harvested seeds used in this experiment. In retrospect the experiment should have been set up earlier, using seed harvested in June or July to better quantify initial dormancy.

Seed from all three populations germinated in tests prior to burial and initial differences in dormancy corresponded well with the recorded degree of fatal germination. In particular, the Perthshire population had high initial germination (low dormancy) and this was associated with a high degree of fatal germination. The differences between populations suggested that there was substantial variation in the extent of initial dormancy in *S. media* seed and that this variance had a genetic component.

It has been shown that burial of *S. media* seed results in development of a light requirement for seed germination (Wesson & Wareing, 1969b; Froud-Williams, Drennan & Chancellor, 1984) and that buried seeds can be very sensitive to low levels of light, such as those given by a green safe lamp (Baskin & Baskin, 1979). Seeds in this experiment were buried at both 5 cm and 10 cm. As light rarely penetrates more than 0.5 – 1.0 cm into soil (Egley, 1995), fatal germination of buried seed occurred in the absence of light. However fatal germination of the Leicestershire and Caithness populations was less than might be expected from the extent of initial germination. Given that initial germination was tested following a short exposure to red light (and subsequent intermittent exposure to low levels of green light), this suggests that some seeds from the Leicestershire and Caithness populations had a pre-determined light requirement for germination, or that this was developed during burial (Kryger Jensen, 1995). Although initial germination tests were not conducted in light and darkness to determine whether this light requirement was genetically determined or induced by burial, the genetic argument is supported by observations showing that germination of the Leicestershire population was particularly promoted by an initial exposure to red light (Chapter 6). Chapter 6 also showed that germination of the Perthshire population was

insensitive to light and therefore the absence of light at depth in soil would not have prevented germination, given that other conditions were suitable.

5.5.2. Seasonal changes in dormancy

Seasonal changes in dormancy were evident in germination tests with exhumed seeds (Figure 5.2). The general pattern of seasonal change in dormancy was common to all three populations, with release of initial dormancy over winter and rapid dormancy induction in early summer. Differences between populations were recorded for germination behaviour at 30 °C and for patterns of dormancy induction in autumn 1999.

This dependence on test conditions for demonstrating dormancy has been emphasised by Bouwmeester & Karssen (1992, 1993a, b, c) and Milberg & Andersson (1997). Expression of seasonal changes in dormancy showed some dependence on temperature in the germination test, though notably only at 30 °C and including a greater range of temperatures may have better defined this relationship. However even with the limited data presented, it is clear for the Perthshire and Caithness populations that dormancy induction was evident in germination tests at 30 °C, earlier than that recorded at lower temperatures (Figure 5.2). Dormancy induction with an initial decrease in germination at higher temperatures has also been recorded for the winter annual species *Lamium amplexicaule* and *L. purpureum* (Baskin, Baskin & Parr, 1986) and *Viola arvensis* (Baskin & Baskin, 1995). Results from these experiments also showed high levels of dormancy during the summer months.

Negligible germination was recorded for the Leicestershire population at 30 °C and as such early dormancy induction was not evident. It is not clear why this population largely failed to germinate at 30 °C, but relatively low levels of germination have been recorded at this temperature in other experiments (Chapters 6, 8 & 10).

There was marked variation between populations for patterns of dormancy release in August and September 1999 (Figure 5.2). Increasing germination suggested that dormancy relief was occurring, but at different rates for the different populations. The expectation was for dormancy to continue to decline, with increased germination over a wider range of test temperatures. This would then complete the seasonal dormancy cycle. However in October 1999, low levels of germination were recorded for all populations and at all temperatures. For the Caithness population this reflected a small increase in the proportion of seeds germinating from the low levels recorded in September 1999 and as such it could be argued

that dormancy relief was continuing, albeit slowly. For the Leicestershire and Perthshire population this was more problematic as this represented a large-scale change from low to high levels of dormancy.

Observation of soil temperature data for September and October 1999 did not indicate that there had been any marked changes from seasonal averages. Seed viability was markedly reduced in September 1999, but not in October 1999 (Figure 5.4) and whilst this may question the reliability of the September 1999 data, it does not explain reduced viable seed germination in October 1999. It should be noted that Crop Division technical staff set up and recorded the September and October test series (with the exception of seed viability). Identical procedures for seed collection and germination were followed and there was no suggestion of experimental error.

It therefore remains unclear why germination decreased markedly for the Leicestershire and Perthshire populations in October 1999 and it is unfortunate that a longer time series of exhumations was not planned. In retrospect, the interval between exhumations should have been extended or additional seed samples should have been buried in order to collect data for a more extended time series.

5.5.3. Seasonal changes in the proportion of viable seeds

There were marked differences in viable seed germination and the proportion of viable seeds between samples recovered from the two blocks. This was despite a maximum of 5 metres separation between blocks. The difference was most marked in April 1999 and comparison of Figure 5.1 and Figure 5.3 shows that the marked reduction in viable seed germination was associated with a similar decline in the proportion of viable seeds recovered at the end of the germination test. This large-scale decline in seed germination and seed viability was recorded for all three populations with similar severity. From observation of the non-viable seeds identified on completion of the germination tests, it was considered that there was fungal infection of these seeds. Given that this infection was restricted to seed samples from block 1 and occurred in all test samples, it was evident that this infection was soil transmitted. If block 1 results from April 1999 were excluded, viable seed germination from October 1998 to August 1999 inclusive corresponded closely to that recorded in block 2.

The proportion of viable seeds recovered at the end of the germination test also decreased markedly in block 2 in September 1999 (Figure 5.4). All three populations were again affected, but effects on the Caithness population were particularly severe and there was a suggestion that seed viability decreased with increasing test temperature. The loss of viability again appeared to be associated with a soil-borne fungal infection, although the physical characteristics of the infection were different and there was little evidence of an effect on viable seed germination, except for the Caithness population.

Overall there appeared to be a suggestion that the proportion of viable seeds recovered decreased from August 1999 onwards. This was evident in both blocks and for all populations. Given that efforts were made to restrict germination tests to viable seeds, this overall decline may reflect increasing low level fungal or bacterial infection as accumulated with increasing length of burial. It may also correspond to published rates of annual decline in buried seed viability, variously estimated at 19 % (Roberts & Feast, 1973) and 30 % (Roberts & Dawkins, 1967 in Sobey, 1981).

5.5.4. Implications for *S. media* control

The high level of fatal seed germination for seed produced and buried in late summer/early autumn suggests that delayed inversion tillage may be effective in reducing seedling emergence from the *S. media* seedbank. However utilising low levels of 'innate' seed dormancy to maximise fatal seed germination in the autumn may be problematic as this ignores the fact that *S. media* forms a persistent seed bank. Therefore although this may limit emergence from current seeds, the soil seedbank will likely include seed ranging in age and germination requirements. It also fails to recognise that with high seed output, a proportionally small dormant seed fraction could still pose a significant weed problem. Finally although fatal seed germination can be substantial, there is significant variation between populations and, allowing for the other problems identified, this approach may only limit emergence for populations exhibiting low dormancy and no light requirement for germination.

Greater significance for weed control is attributed to the detrimental effect that some soil borne fungi may have in reducing seed viability. High levels of fungal infection were evident on two occasions and in one case, in addition to reducing the proportion of viable seeds recovered, it was likely that low level infections reduced 'viable' seed germination.

Given the efficacy of infection against all populations, the use of fungi in biological control of *S. media* seeds would merit further investigation. Commercial mycoherbicides have been developed for other weed species, as described in Cousens & Mortimer (1995).

5.6. Summary

Initial dormancy varied between populations and where seed dormancy was low, high levels of fatal seed germination were recorded, particularly for the Perthshire population. It was thought that this was compounded by absence of a light requirement for germination in this population (section 6.4).

Seasonal changes in seed dormancy were largely similar for all three populations. Dormancy release in the autumn widened the range of temperatures at which seeds germinated, initially restricting germination to temperatures less than 30 °C. Dormancy induction in the spring narrowed the range of temperatures at which seeds germinated, increasingly restricting germination to temperatures less than 30 °C. Patterns of dormancy release in the second autumn were ill-determined, largely by lack of extended time-series data.

Seed viability generally decreased over the year-long burial, but significantly two periods of suspected soil borne fungal infection dramatically reduced seed viability. This demonstrated the importance of blocking in seed burial experiments. It more importantly suggested an option for developing biological control methods for *S. media*.

Chapter 6. The effect of temperature, water potential and light on seed germination in contrasting populations of *Stellaria media*.

6.1. Summary

An experiment was conducted to investigate the effect of temperature, water potential and light on the germination time courses of three contrasting populations of *Stellaria media*. The data were then used to assess the utility of using the hydrothermal time model (Gummerson, 1986) to describe germination patterns for *S. media*.

Final percentage germination and the cumulative germination time courses differed significantly between populations and according to constant temperature, water potential and light conditions at initiation. In general, final percentage germination was maximised at 10 °C in water and following an initial exposure to red light. Lower water potentials tended to decrease final percentage germination. The time to the onset of germination tended to decrease with increasing temperature up to an optimal 20 °C and then increased at higher temperatures. It also increased with lower water potentials. Germination tended to be most synchronous at 20 °C in water. There were exceptions and the final percentage germination of the Caithness population was higher at 30 °C than 20 °C and was not affected by low water potentials. The Leicestershire population was also notably sensitive to light conditions at initiation.

Application of the hydrothermal model was problematic. Although in general the start of germination tended to be reasonably well fitted, the speed of germination was poorly predicted. The fits of the hydrothermal model also overpredicted final percentage germination and this was largely through failure to reach an asymptote. It is shown that this problem is inherent in the structure of the hydrothermal time model and this together with difficulties in estimating confidence intervals for model output and parameter values undermine the utility of this method. Alternative models for the data are developed and discussed in Chapter 11.

6.2. Introduction

Stellaria media dormancy has been shown to vary seasonally with changes in soil temperature (Chapter 5) and it is clear that these changes may markedly affect the proportion of *S. media* seeds germinating following cultivation at different times of the year. However, the actual number of seeds germinating following cultivation will depend on the rate and timing of germination and as such, modelling of weed seedling emergence requires both a dormancy model to describe how seed germination requirements vary seasonally and a model to describe the rate and timing of germination, according to prevailing environmental conditions.

As described in Chapter 4, Vleeshouwers (1997) proposed a model of weed seedling emergence that separated the processes of seed dormancy, seed germination and seedling emergence. This was developed for summer annual weed species with a light requirement for germination. In the germination model, the timing of seed germination was simulated by a thermal time model (see explanation below). This followed from the fact that temperature has long been recognised as an important factor in determining the timing and duration of flushes of weed seedlings (Hegarty, 1973, Bouwmeester & Karssen, 1992). However, soil water potential has also been shown to have an important effect (Roberts, 1984) and this was not included in the Vleeshouwers model. Vleeshouwers (1997) accepts that this is a limitation, restricting application of this model to periods when soil moisture is not limiting. Broader application of this and similar models requires that data are available to quantitatively describe the effect of reduced water potentials on weed seed germination. An approach that combines the effect of temperature and water potential on the timing of germination has been developed for crop plants using a concept of hydrothermal time and this is explained below.

Thermal time models were developed to quantify the influence of temperature on the rate of seed germination (Hegarty, 1973; Garcia-Huidobro, Monteith & Squire, 1982). These models follow from the observation that, in germination experiments using a range of constant temperatures, a plot of germination rate versus temperature is linear at sub-optimal temperatures. If $t(g)$ is the time to completion of germination for fraction g, then

$$1/t(g) = [T - T_b(g)]/\theta_T(g) \qquad (6.1)$$

where $1/t(g)$ is the germination rate for cumulative fraction g, T is the temperature, $T_b(g)$ is known as the base temperature for fraction g and the slope is $1/\theta_T(g)$, again for fraction g. The fraction g can be defined as a proportion of either the total number of seeds or the total number of germinable seeds in an experiment, but the definition used is not always explicitly stated (e.g. Grundy, 1997). With few exceptions, it has been shown that there is little variation in $T_b(g)$ with germination fraction and therefore a common base temperature T_b can be identified (Garcia-Huidobro, Monteith & Squire, 1982, Ellis, Simon & Covell, 1987). However, it should be noted that exceptions have occurred in some cases where there was seed dormancy (Bradford, 1995). Accepting this assumption that T_b is constant for all seeds and rearranging equation (6.1)

$$\theta_T(g) = (T - T_b)\,t(g) \qquad (6.2)$$

and $\theta_T(g)$ can be considered to be the thermal time requirement for germination of fraction (g).

Gummerson (1986) extended this to a concept of hydrothermal time. The hydrothermal time model attempts to define a single equation that predicts the time course of germination over a range of temperatures and water potentials. For suboptimal temperatures this equation was defined as:

$$\theta_{HT} = (\psi - \psi_b(g))\,\theta_T(g) \qquad (6.3)$$

where θ_{HT} is the hydrothermal time constant, ψ is the water potential and $\psi_b(g)$ is the base water potential that just prevents germination of fraction g. The reason that θ_{HT} is a constant will be explained in the results section.

Although this concept has been successfully applied to a number of crop plants (Gummerson, 1986, Dahal, Bradford & Jones, 1990; Dahal & Bradford, 1990) to date application to weed plants has been limited to one population of *Stellaria media* (Grundy, 1997) and this was with questionable success. Grundy (1997) presented data on the influence of temperature and water potential on the germination of seven dry seed lots of *S. media* and applied the hydrothermal model to a three year old seed lot of *S. media*, selected specifically because it had the lowest dormancy. Grundy (1997) concluded that the

hydrothermal time concept could estimate the germination rate between 10 and 20 °C, but that further work was required to define the relationships more precisely, especially at lower temperatures. Moreover, as the model was only applied to one seed lot (because of difficulties in comparing seed lots of different ages, derived from different places) it was impossible to assess the extent to which different seed lots varied in terms of the identified parameters in the hydrothermal model. A further criticism is related to lack of control in the light environment, whereby seeds were intermittently exposed to diffuse light during recording. *S. media* is a winter annual, widely reported as a species that requires light to germinate, especially following seed burial (Wesson & Wareing, 1969 a & b).

Following the work of Grundy (1997), experiments were set up to compare the influence of temperature and water potential on the germination of three equal age seed populations of *S. media*, under controlled light conditions. The three populations used were the contrasting populations selected in Chapter 2. The investigation of seed response to light involved the comparison of seeds intermittently exposed to very low levels of green light and seeds initially exposed to red light and then intermittently exposed to very low levels of green light. Following Baskin & Baskin (1979) it is acknowledged that the use of a green safe light cannot be assumed to represent a dark treatment. However it should be noted that Baskin & Baskin (1979) recorded *S. media* germination following exposure to a green safe light for seeds previously buried for 16 or 18 months, where it is known that burial induces a light requirement for germination. The seeds used in this experiment were previously dry-stored after harvest. The green safe light used by Baskin & Baskin (1979) also provided notably higher light levels than those used in this experiment, with PPFD of between 3.7 and 5.7 $\mu mol\ m^{-2}\ s^{-1}$.

6.3. Methods

6.3.1. Seed used

The experiment used mature seeds that were harvested in August 1996. The seed was harvested from plants grown in an unheated glasshouse with seed set under muslin to prevent cross-pollination (as described in section 2.3.2). Seeds were stored dry in an incubator maintained at 10 °C (± 2 °C) prior to the experiment.

6.3.2. Experimental design and treatments

In February 1998, experiments were established to assess the germination time course of the three populations in all combinations of four constant temperatures (5, 10, 20 and 30 °C) and three water potentials (0, -0.4 and –0.8 MPa), with or without exposure to red light at initiation. All seeds were subsequently exposed to intermittent very low levels of green light.

For each germination test, 50 seeds were placed on a double layer of filter paper (Whatman no. 181) in a 9 cm plastic Petri-dish. There were three replicates for each treatment, established at weekly time intervals to counter the lack of replication in incubators. Solutions differing in water potential were prepared using polyethylene glycol 8000 (PEG), with different concentrations of PEG used for each temperature and water potential combination as described by Michel (1983). The PEG solutions were prepared in bulk. Under a green safe light, each Petri dish received 10 ml of the appropriate PEG solution or distilled water and was then sealed in a self-seal clear polythene bag, to minimise changes in water potential due to evaporation. The green safe light was given by two Osram L 36W/30 fluorescent tubes with diffusers covered with two layers of dark yellow-green Cinemoid filter (Lee filters #90), giving a photosynthetically active photon flux density (PPFD) of 0.51 $\pm$ 0.19 μmol $m^{-2} s^{-1}$. Petri-dishes that were not exposed to red light were removed to sealed cardboard boxes wrapped in black polythene and lined with bubble wrap. The seeds that were given a red light treatment were then laid out for 15 minutes beneath two Osram L 36W/30 fluorescent tubes with diffusers covered with two layers of bright red Cinemoid filter (Lee filters #26), giving a PPFD of 3.84 $\pm$ 0.71 μmol $m^{-2} s^{-1}$ (PPFD recorded by quantum sensor and a Campbell CR10 datalogger). Under the green safe light, these Petri dishes were then randomly placed in the same sealed cardboard boxes, with a separate box for each temperature. The boxes were then removed to unlit incubators maintained at the defined constant temperatures ($\pm$ 2 °C). Germination was assessed under the green safe light, daily for the first ten days (twice daily on the second day when the peak of germination at 20 °C was observed) and then at less frequent intervals until germination ceased. Seed germination was defined as radicle emergence to greater than 1 mm and germinated seeds were removed from Petri dishes when recorded.

6.3.3. Initial data analysis

To summarise the data, separate Gompertz curves were fitted to cumulated seed germination counts for each Petri dish. The Gompertz curve relates the cumulative germination fraction, g to time, t, as follows:

$$g = \alpha + \gamma \exp(-\exp(-\beta(t-\mu))) \qquad (6.4)$$

where the parameter β is related to the slope of the curve and larger values of β are associated with greater synchronicity of germination. The parameter μ equals the time to the point of inflexion in the curve and as such represents the timing of the germination. The parameters α and γ define the asymptotes of the fitted curve and the sum of these parameters represents the total number of seeds germinated for each population.

Statistical analysis of population and treatment effects used analysis of variance (Genstat 5) to compare the fitted Gompertz parameters and derived estimates of mean percentage germination. Data sets that were poorly fitted by the Gompertz curve ($r^2 < 0.8$) were excluded from the analysis.

6.3.4. Calculation of base temperatures and application of the hydrothermal model

As outlined in the introduction, thermal and hydrothermal time (equations (6.2) and (6.3)) can be used as the basis for a predictive model for seed germination in relation to temperature and water potential. The first step is to estimate the common base temperature (T_b) in water.

Base temperature can be calculated by two different methods, each based on identifying a relationship between germination in water and temperature at sub-optimal temperatures. Sub-optimal temperatures were defined as less than or equal to the temperature associated with the maximum observed germination rate.

The simplest method is to first calculate germination rates as the reciprocal of the time taken for a fraction (g) of the total seed population to germinate, $1/t(g)$. This can be done by solving the fitted Gompertz equations (6.4) to give a range of $t(g)$ for each data set. The germination rate for different seed fractions (calculated as deciles) can then be plotted against sub-optimal temperatures and linear regression used to define relationship between

the two variables, according to equation (6.1). The base temperature is then estimated by extrapolating the regression line and solving for germination rate = 0. The problem with this method is that separate regression lines have to be fitted for each seed fraction and where there is variation in the estimate of T_b, authors have then had to nominally identify a single base temperature (generally calculated from $1/t(50)$, e.g. Grundy (1997)).

Secondly, an alternative method estimates a single value of T_b by combining all data from germination time courses at a range of sub-optimal temperatures in a common regression using probit analysis.

$$probit\,(g) = \frac{[(T - T_b)\,t(g)] - \theta_T(50)}{\sigma_{\theta_T}} \qquad (6.5)$$

where *probit* (g) is the probit transformation of percentage g (which linearises a cumulative normal distribution (Finney, 1971)). Equation (6.5) assumes that the thermal time required for germination is normally distributed with $\theta_T(50)$, the median thermal time to radicle emergence and σ_{θ_T}, the standard deviation in thermal time requirements among seeds in the population (the inverse of the slope of the probit regression line). T_b is estimated by repeated probit regression analysis, using different values of T_b in order to identify the value of T_b that minimises the residuals of the probit regression line (6.5). Having identified a base temperature, equation (6.5) can then be used to predict germination time courses at different temperatures in water.

In an attempt to calculate confidence intervals for the probit estimated base temperatures, bootstrap resampling (Efron & Tibishirani, 1993) was used. The original data set consisted of individual germination time courses. The bootstrap estimation involved constructing new data sets of the same size as the original by random resampling (with replacement) of the individual germination time courses. The base temperature was then estimated as described above, using this new data set and the process repeated many times. This established a frequency histogram of base temperature values which was then used to determine approximate confidence intervals.

To extend the application to consider different water potentials, the hydrothermal model was developed. It might be assumed that an analogous approach to that used for thermal time should be adopted, whereby the hydrothermal time (equation (6.3)) required for germination

is normally distributed among the seed population. In this case, both T_b and the base potential, ψ_b, are constants. T_b is determined from equation (6.5) as described above and the base potential, ψ_b, is similarly calculated from repeated probit regression analysis according to equation (6.6) below:

$$probit(g) = \frac{\theta_{HT} - \theta_{HT}(50)}{\sigma_{\theta_{HT}}} \tag{6.6}$$

However this approach often results in a poor fit to the data (Ellis, Covell, Roberts & Summerfield, 1986; Ellis, Simon & Covell, 1987) and this was echoed in analysis of this experiment (results not shown). Instead, the approach adopted by Gummerson (1986) assumes that the hydrothermal time required for germination is constant, but that the base water potential, ψ_b, varies. Assuming that the base water potential, ψ_b, is normally distributed within the seed population with mean $\psi_b(50)$, and standard deviation, σ_{ψ_b}, gives equation (6.7)

$$probit(g) = \frac{\psi_b(g) - \psi_b(50)}{\sigma_{\psi_b}} \tag{6.7}$$

where $\psi_b(g)$ is given by rearranging equation (6.3)

$$\psi_b(g) = \psi - \frac{\theta_{HT}}{(T - T_b)\,t(g)} \tag{6.8}$$

For each combination of water potential, ψ, temperature, T, and time, $t(g)$, we have a germination fraction (g). Using the appropriate base temperature as described above, and a given value for the hydrothermal time constant, θ_{HT}, the parameters in equation (6.7) can be determined by regression. The optimal hydrothermal time constant is estimated by further repeated probit regression analysis, using different values of θ_{HT} in order to identify the value of θ_{HT} that minimises the residuals of the probit regression line (6.7). Probit analysis, bootstrap estimation and fitting of the hydrothermal time models used purpose written routines (Dr. Glenn Marion) within the statistical package R (http://www.ci.tuwien.ac.at/R/). It should be noted that all available data were used to fit the models and no independent

model validation is presented. As such r^2 statistics describe model goodness of fit and are likely biased estimates of the models predictive ability.

6.4. Results

The Gompertz distribution was fitted to cumulated counts of germinated seeds for each Petri dish where seeds germinated. No germination was recorded in 11 Petri dishes, including all three replicates of the Leicestershire population at 5 °C in -0.8 MPa PEG without light stimulus at initiation. The percentage variance (r^2) accounted for by the fitted Gompertz curves exceeded 90 % in 197 of the remaining 205 data sets and 80 % in all but one replicate of the Perthshire population at 30 °C in -0.8 MPa PEG and with red light on initiation. This one data set was also excluded from further analysis.

6.4.1. Percentage germination

Table 6.1 shows the mean percentage germination of *S. media* seeds after 32 days for each of the three populations according to treatment. For all treatments, no substantial germination was recorded after 25 days. Table 6.1 and Table 6.4 (summary of analysis of variance) show that for each population, the number of seeds germinating decreased as water potential decreased, with the exception of the Caithness population at 30 °C. Percentage germination was significantly higher for the Perthshire population in all treatments, except at 30 °C and especially at the lower water potentials where germination was higher for the Caithness population. Percentage germination tended to be highest at 10 °C and lowest at 5 and 30 °C, depending on water potential. At low water potentials (with the exception of the Caithness population), germination was more markedly reduced at 30 °C than 5 °C. Exposure to red light at initiation of the experiment had a significant effect on percentage germination with more seeds germinating following exposure to red light. This effect was most evident for the Leicestershire population and least evident for the Caithness population. For the Caithness population there was no significant effect of light at 20 °C and 30 °C.

Population	Water potential (MPa)	+Light				-Light			
		5 °C	10 °C	20 °C	30 °C	5 °C	10 °C	20 °C	30 °C
Leicestershire	0	42.26	58.77	62.63	44.08	18.97	32.80	27.05	9.50
	-0.4	36.32	42.85	33.98	16.91	13.74	12.44	9.50	2.04
	-0.8	10.74	33.25	28.95	2.82	0.00	9.56	11.46	0.68
Perthshire	0	88.19	96.38	91.74	87.76	70.58	91.00	81.10	81.28
	-0.4	66.83	78.84	65.53	26.54	40.51	61.33	58.54	22.57
	-0.8	23.54	42.62	27.58	2.27	14.10	25.08	12.10	1.40
Caithness	0	23.19	41.42	29.57	48.57	19.58	27.64	26.10	57.34
	-0.4	16.81	25.88	12.69	33.04	4.71	12.52	12.80	31.77
	-0.8	5.48	11.98	2.04	39.05	2.68	6.14	2.74	40.24

Table 6.1: Mean percentage germination of *Stellaria media* seeds at different combinations of temperature and water potential, with or without red light at initiation as estimated by summing Gompertz parameters ($\alpha + \gamma$) and including zero values for samples where no germination was recorded

6.4.2. Germination time

Table 6.2 shows the mean value of the Gompertz parameter μ (the time to the point of inflexion of the Gompertz curve) for the same *S. media* seed populations and treatments. Table 6.2 and Table 6.4 (summary of analysis of variance) show that for each population, the time to the point of inflexion (related to time of germination) increased as water potential decreased. The time to inflexion also interacted with temperature, increasing as temperatures both decreased from 20 to 5 °C and increased from 20 to 30 °C. The time to inflexion tended to be shortest at 20 °C. Exposure to red light at initiation also tended to shorten the time to inflexion and again this effect was most evident for the Leicestershire population (not significant ($P > 0.05$) for the Perthshire and Caithness populations). Considering all treatments, time to inflexion was shortest for the Leicestershire population and longest for the Caithness population. Table 6.4 showed that the temperature associated with the shortest time to inflexion interacted with water potential. In distilled water the time to inflexion was shortest at 30 °C, whereas at lower water potentials, the time to inflexion tended to be shorter at 20 °C.

Population	Water potential (MPa)	+Light				-Light			
		5 °C	10 °C	20 °C	30 °C	5 °C	10 °C	20 °C	30 °C
Leicestershire	0	197	118	40	37	217	119	46	40
	-0.4	238	141	58	67	291	145	88	128
	-0.8	349	193	77	140	*	211	76	231
Perthshire	0	207	117	40	37	209	123	39	37
	-0.4	254	159	68	68	285	160	59	73
	-0.8	328	199	73	431	338	201	71	221
Caithness	0	213	116	52	46	221	121	54	42
	-0.4	251	160	81	70	277	167	68	74
	-0.8	349	250	113	129	488	204	183	110

Table 6.2: Mean Gompertz parameter μ (time to inflexion point, hours) for *Stellaria media* seeds at different combinations of temperature and water potential, with or without red light at initiation.
*No germination recorded.

6.4.3. Synchronicity of germination

Table 6.3 shows the mean value of Gompertz parameter β for the same *S. media* seeds according to population and treatment. Table 6.3 and Table 6.4 (summary of analysis of variance) show that for each population, water potential and light treatment combination, germination was least synchronous at 5 °C. There was a significant trend to higher synchronicity of germination in the Leicestershire population and also a general pattern of decreasing synchronicity of germination with decreased water potential. Table 6.4 showed that the temperature that maximised the synchronicity of germination interacted with the test water potential. In distilled water the synchronicity of germination was highest at 10 °C and at lower water potentials, germination was more synchronous at either 20 or 30 °C. Exposure to red light at initiation did not significantly effect the synchronicity of germination.

Figure 6.1, Figure 6.2 and Figure 6.3 show fitted Gompertz curves according to temperature, water potential and light for each population.

Population	Water potential (MPa)	+Light				-Light			
		5 °C	10 °C	20 °C	30 °C	5 °C	10 °C	20 °C	30 °C
Leicestershire	0	0.043	0.083	0.053	0.053	0.027	0.080	0.053	0.053
	-0.4	0.040	0.053	0.057	0.060	0.027	0.073	0.067	0.063
	-0.8	0.027	0.050	0.067	0.067	*	0.043	0.063	0.050
Perthshire	0	0.030	0.053	0.053	0.053	0.033	0.057	0.050	0.053
	-0.4	0.023	0.037	0.060	0.053	0.030	0.047	0.057	0.067
	-0.8	0.030	0.053	0.063	0.010	0.027	0.040	0.057	0.060
Caithness	0	0.037	0.073	0.057	0.057	0.027	0.060	0.053	0.053
	-0.4	0.037	0.060	0.033	0.060	0.030	0.040	0.047	0.060
	-0.8	0.030	0.027	0.075	0.017	0.020	0.040	0.065	0.020

Table 6.3: Mean Gompertz parameter β (synchronicity of germination, hour^{-1}) for *Stellaria media* seeds at different combinations of temperature and water potential, with or without red light at initiation.
* No germination recorded.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	2	6.44	**	1.85	-	366.72	***
Temperature	3	51.37	***	73.32	***	23.95	***
Water potential	2	11.11	***	21.99	***	445.42	***
Light	1	0.36	-	4.48	*	146.99	***
Population x temperature	6	2.69	*	0.93	-	50.93	***
Population x water potential	4	0.66	-	1.30	-	71.55	***
Temperature x water potential	6	9.41	***	1.24	-	5.26	***
Population x light	2	2.39	-	0.54	-	26.68	***
Temperature x light	3	3.19	*	1.50	-	5.13	**
Water potential x light	2	1.18	-	1.55	-	3.38	*
Population x temperature x water potential	12	4.00	***	0.57	-	3.74	***
Population x temperature x light	6	1.93	-	0.84	-	1.12	-
Population x water potential x light	4	1.16	-	0.61	-	2.37	-
Temperature x water potential x light	6	0.92	-	1.20	-	1.13	-
Residual	†143						

(† 11 missing values, except for $\gamma + \alpha$ where zero values included and residual d.f. = 154)

Table 6.4 Summary of the analysis of variance for differences between *Stellaria media* populations for fitted Gompertz parameters according to temperature, water potential and light.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$

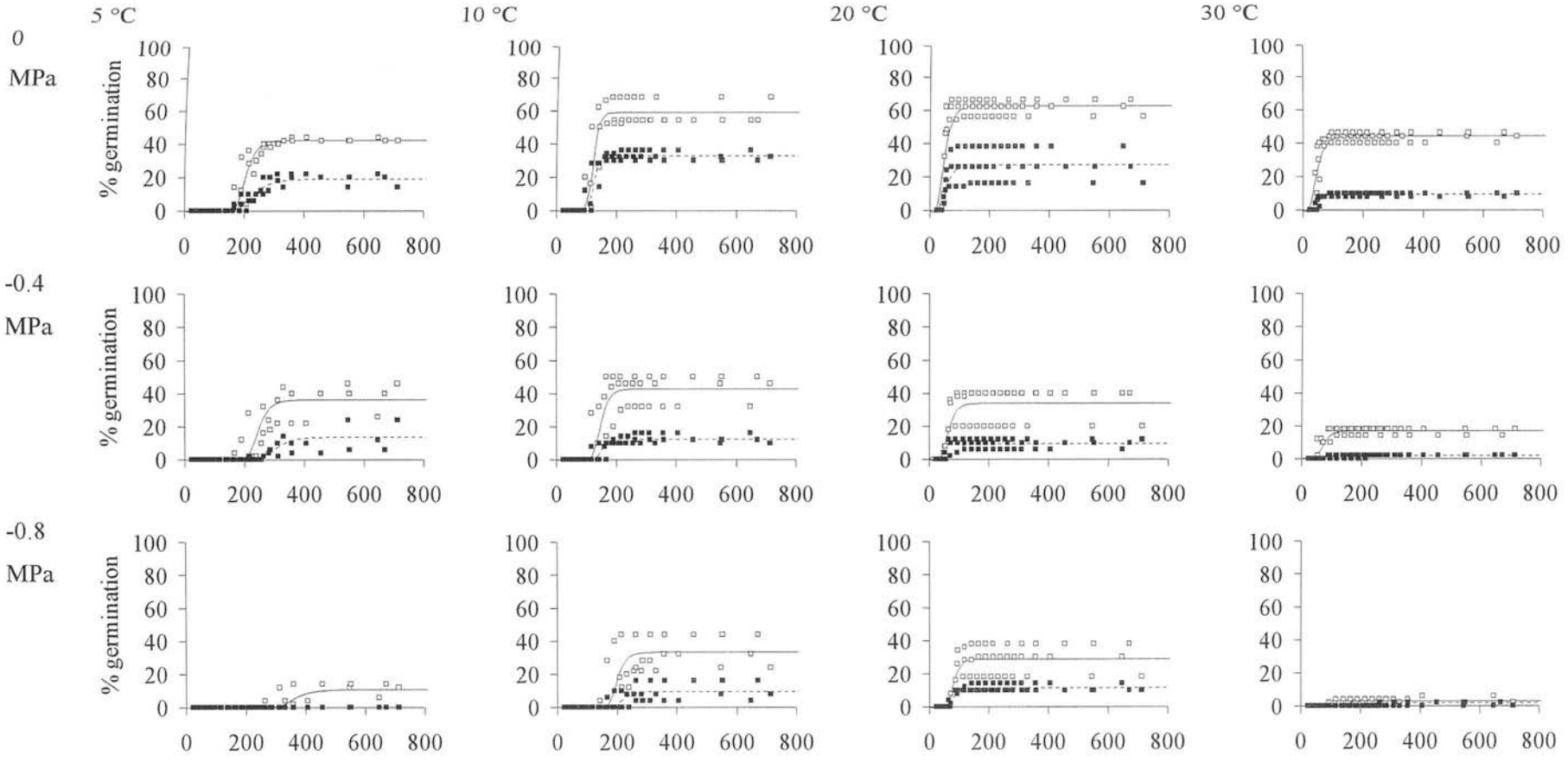


Figure 6.1: Fitted Gompertz curves for germination time courses at 5, 10, 20 and 30 °C and at water potentials of 0, -0.4 and –0.8 MPa for seeds of the Leicestershire population of *Stellaria media* with (open symbols, ___) and without (closed symbols, ----) red light at initiation. n = 3.

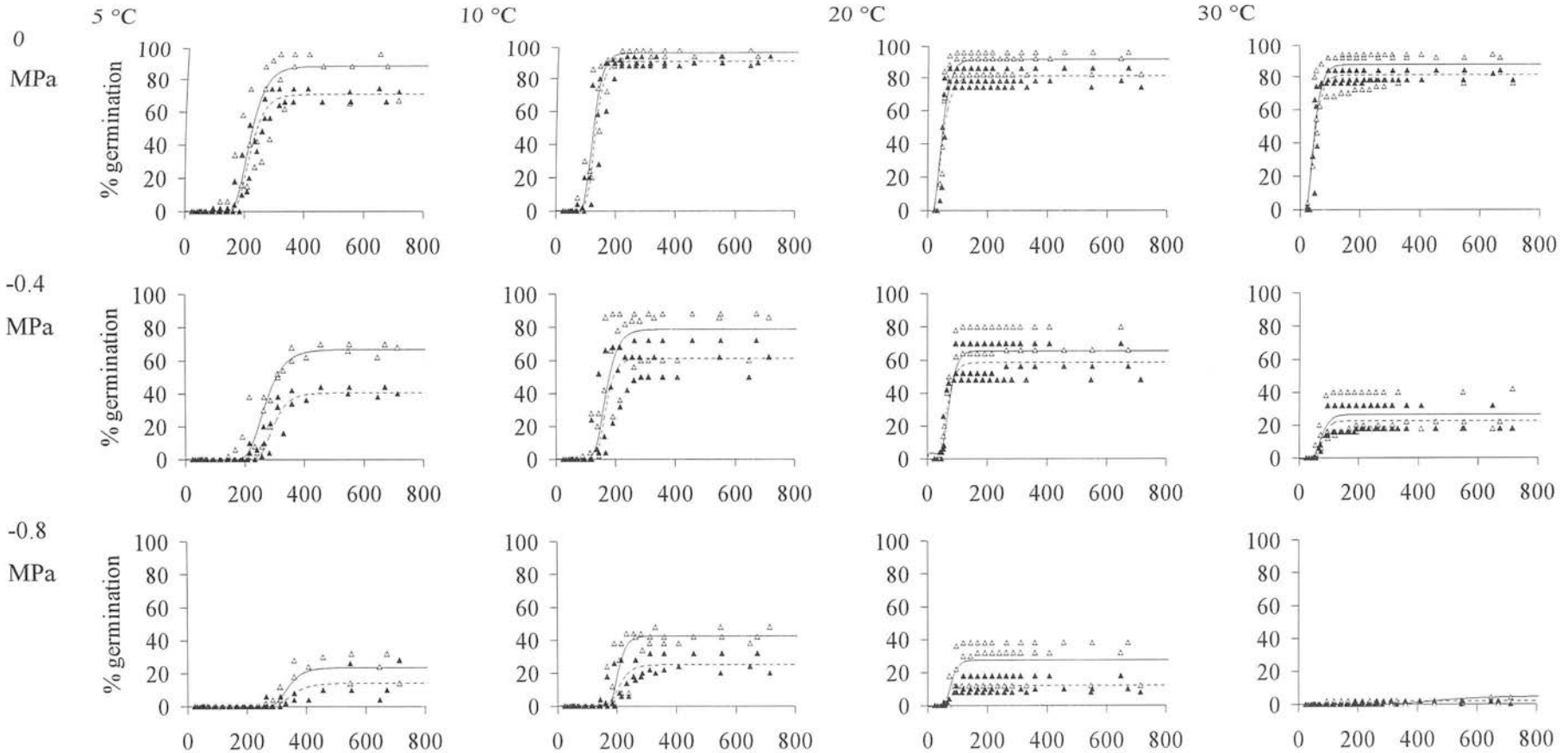


Figure 6.2: Fitted Gompertz curves for germination time courses at 5, 10, 20 and 30 °C and at water potentials of 0, -0.4 and –0.8 MPa for seeds of the Perthshire population of *Stellaria media* with (open symbols, ___) and without (closed symbols, ____) red light at initiation..
n = 3.

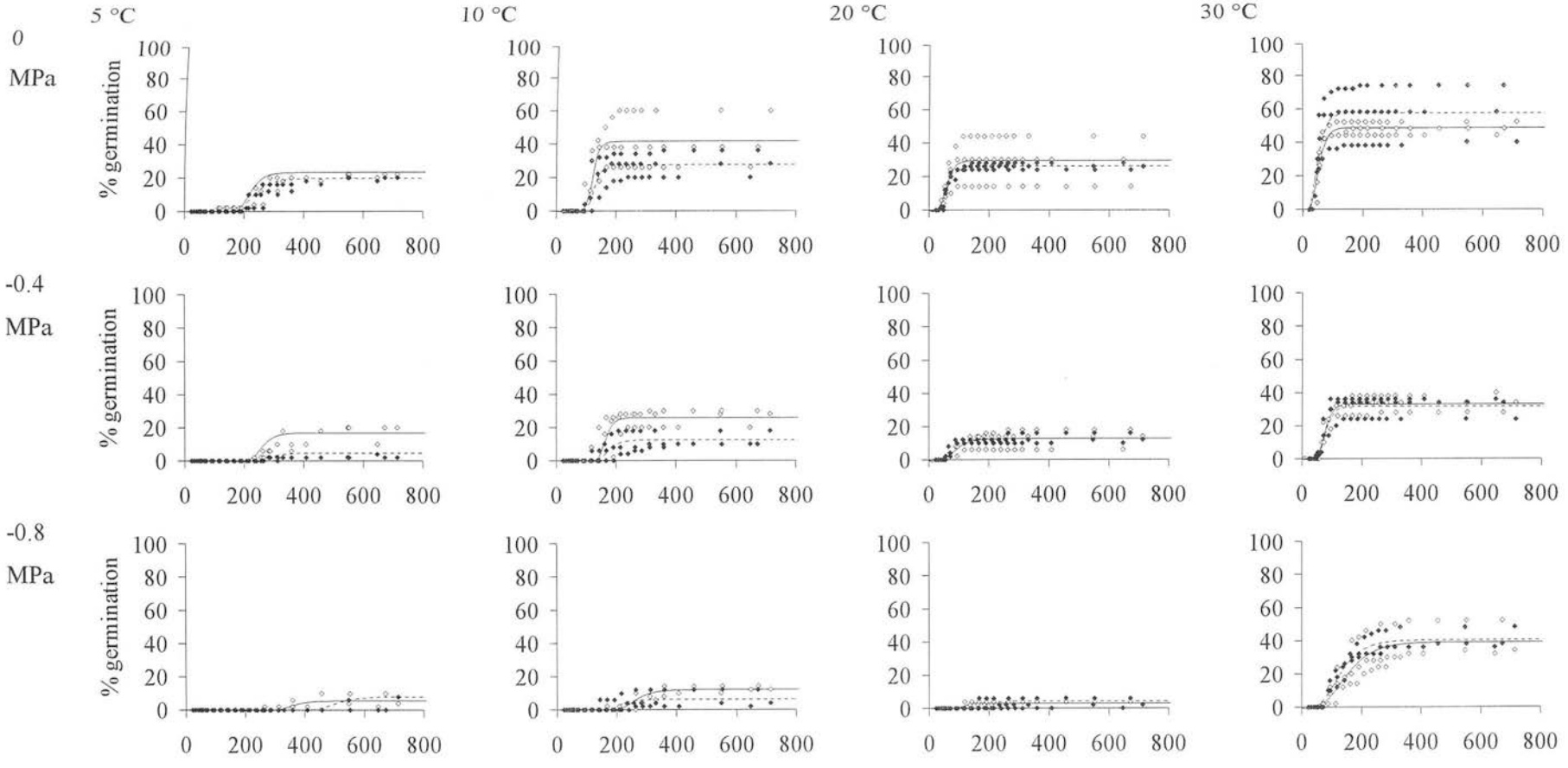


Figure 6.3: Fitted Gompertz curves for germination time courses at 5, 10, 20 and 30 °C and at water potentials of 0, -0.4 and –0.8 MPa for seeds of the Caithness population of *Stellaria media* with (open symbols, ___) and without (closed symbols, ----) red light at initiation. n = 3.

6.4.4. Germination rate and calculation of base temperatures

Table 6.5 details the range of base temperatures estimated from these two methods. Sub-optimal temperatures were identified as 5, 10 and 20 °C, according to Figure 6.4.

Linear regression of germination rates ($1/t(g)$) against temperature in distilled water following exposure to red light for 15 minutes on initiation (Figure 6.4) showed variation in T_b according to germination fraction and variation was highest amongst the lower percentiles, especially at higher temperatures (20 °C). Therefore Table 6.5 presents the estimates from linear regression of $1/t(50)$, or from the rate for the highest germination fraction achieved. For the Caithness population this means that T_b is estimated on the basis of $1/t(20)$ and for the Leicestershire population, in the absence of light, from $1/t(30)$. It is also clear that the linear relationship holds between 5 and 20 °C, but that for the Leicestershire and Perthshire populations, this relationship would break down if the regression line were extended to include 30 °C.

Population	+Light				-Light			
	Probit	95% C.I.	Interpolation		Probit	95% C.I.	Interpolation	
Leicestershire	0.3	-3.1, 3.0	0.5		0.9	-1.7, 2.1	2.1	$t(30)$
Perthshire	2.1	-1.0, 3.2	1.2		1.4	0.2, 2.2	1.0	
Caithness	1.1	-3.1, 2.4	-0.3	$t(20)$	0.8	-0.8, 2.2	3.4	$t(20)$

Table 6.5: Estimated base temperatures (°C) from repeated probit regression analysis (according to equation (6.5)) or from linear regression of germination rate (1/t(50), or highest germination fraction achieved) against temperature and interpolation of T_b. In each case base temperature is estimated from sub-optimal temperatures (5, 10 & 20 °C) in water, with and without exposure to red light for 15 mins on initiation. 95% confidence intervals are estimated by bootstrap resampling.

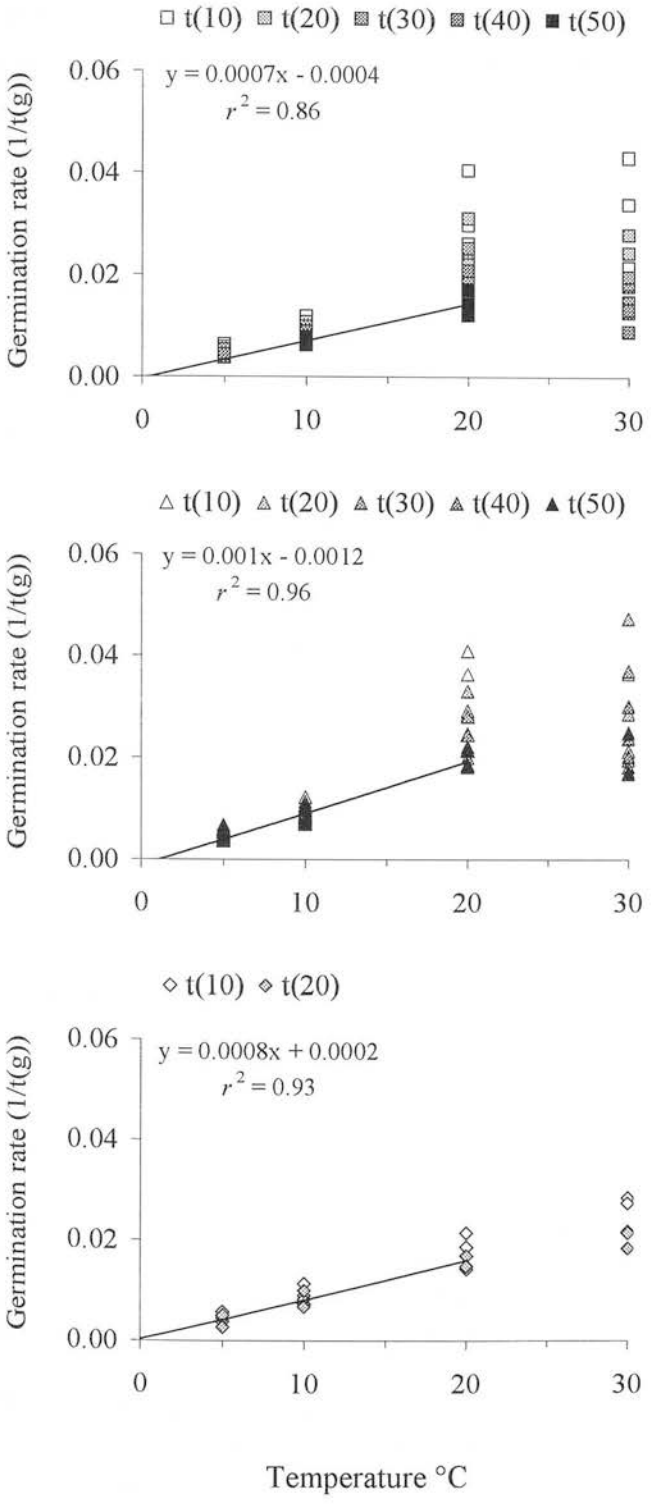


Figure 6.4: The effect of temperature on germination rate ($1/t(g)$) for different cumulative germination fractions of populations of *Stellaria media* in distilled water following exposure to red light for 15 mins on initiation.
n = 3.

It is clear from Table 6.5 that estimates from repeated probit regression analysis (according to equation (6.5)) tended to be less variable between populations and between light conditions than estimates from linear regression of $(1/t(g))$ against temperature. For this reason and because this method estimates a single value of T_b, these values were used to develop the hydrothermal time model. It is worth noting that T_b was positive in all cases except for Caithness population in light and that T_b was consistently higher for the Perthshire population.

6.4.5. Application of the hydrothermal time model

Table 6.6 details the fitted parameters from the hydrothermal time model. Figure 6.5 and Figure 6.6 compare the fitted germination time courses from the hydrothermal time model with experimental results for the Leicestershire population at sub-optimal temperatures with and without red light at initiation. Figure 6.7 and Figure 6.8 show the same comparisons for the Perthshire populations and Figure 6.9 and Figure 6.10 compare the fitted model to seed germination for the Caithness population.

Table 6.6 shows that the fitted parameters varied markedly between populations. The hydrothermal time constant of the Leicestershire population was approximately double that of the Perthshire population in light and decreased when seeds were germinated without light at initiation. Differences between populations for mean base potential and variance were consistent between light treatments.

Population	+Light				-Light			
	T_b	θ_{HT}	ψ_b50	$\sigma_{\psi b}$	T_b	θ_{HT}	ψ_b50	$\sigma_{\psi b}$
	°C	MPa °h	MPa	MPa	°C	MPa °h	MPa	MPa
Leicestershire	0.3	1395	-4.16	0.66	0.9	1085	-4.29	0.85
Pertshire	2.1	595	-2.90	0.41	1.4	735	-2.66	0.38
Caithness	1.1	745	-3.53	0.70	0.8	845	-3.57	0.74

Table 6.6: Estimated hydrothermal time model parameters from repeated probit regression analysis (according to equation (6.7)), for *Stellaria media* populations germinated with and without exposure to red light for 15 mins on initiation.

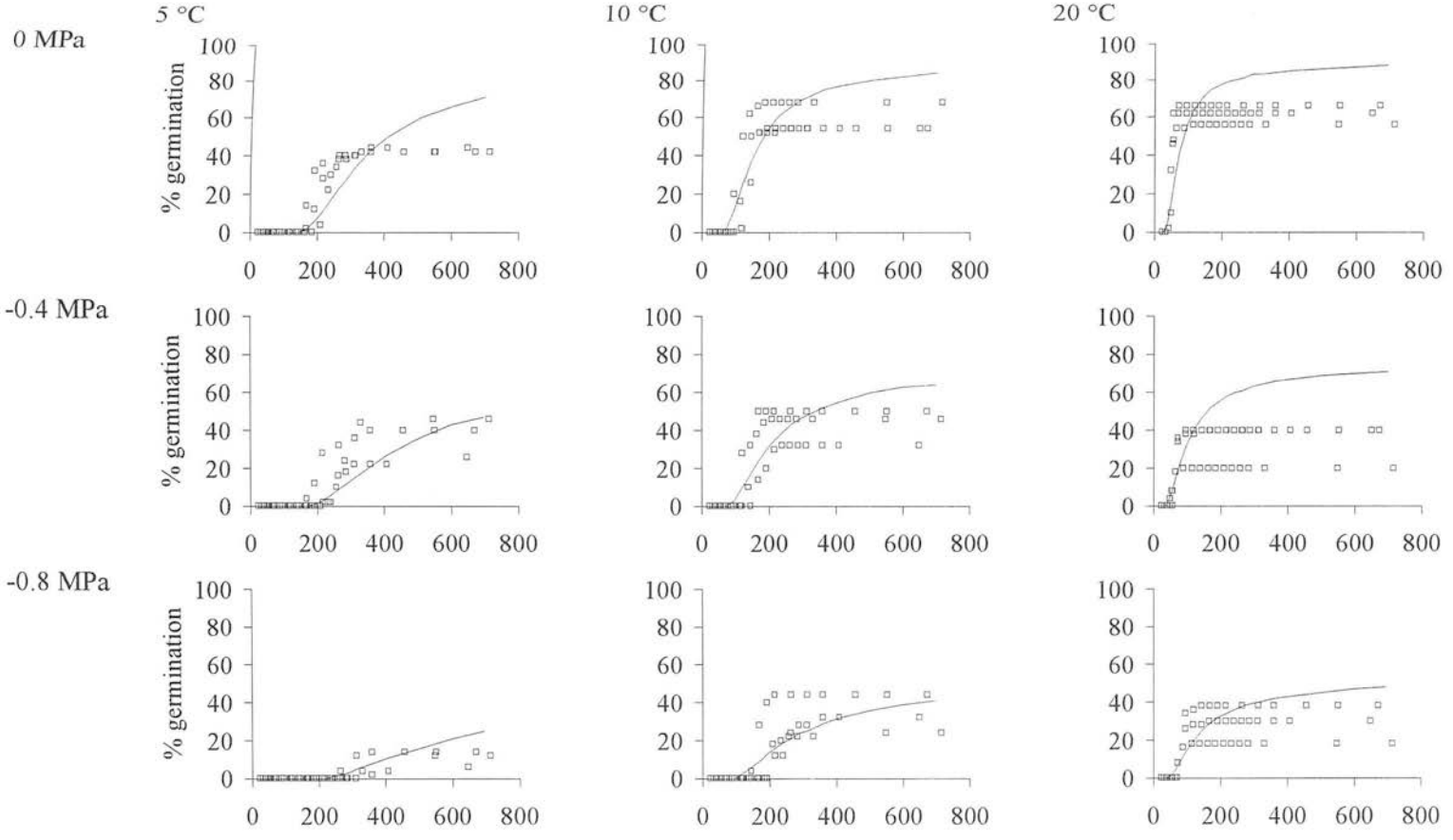


Figure 6.5: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Leicestershire population of *Stellaria media* at sub-optimal temperatures with red light at initiation. $r^2 = 0.56$.
n = 3.

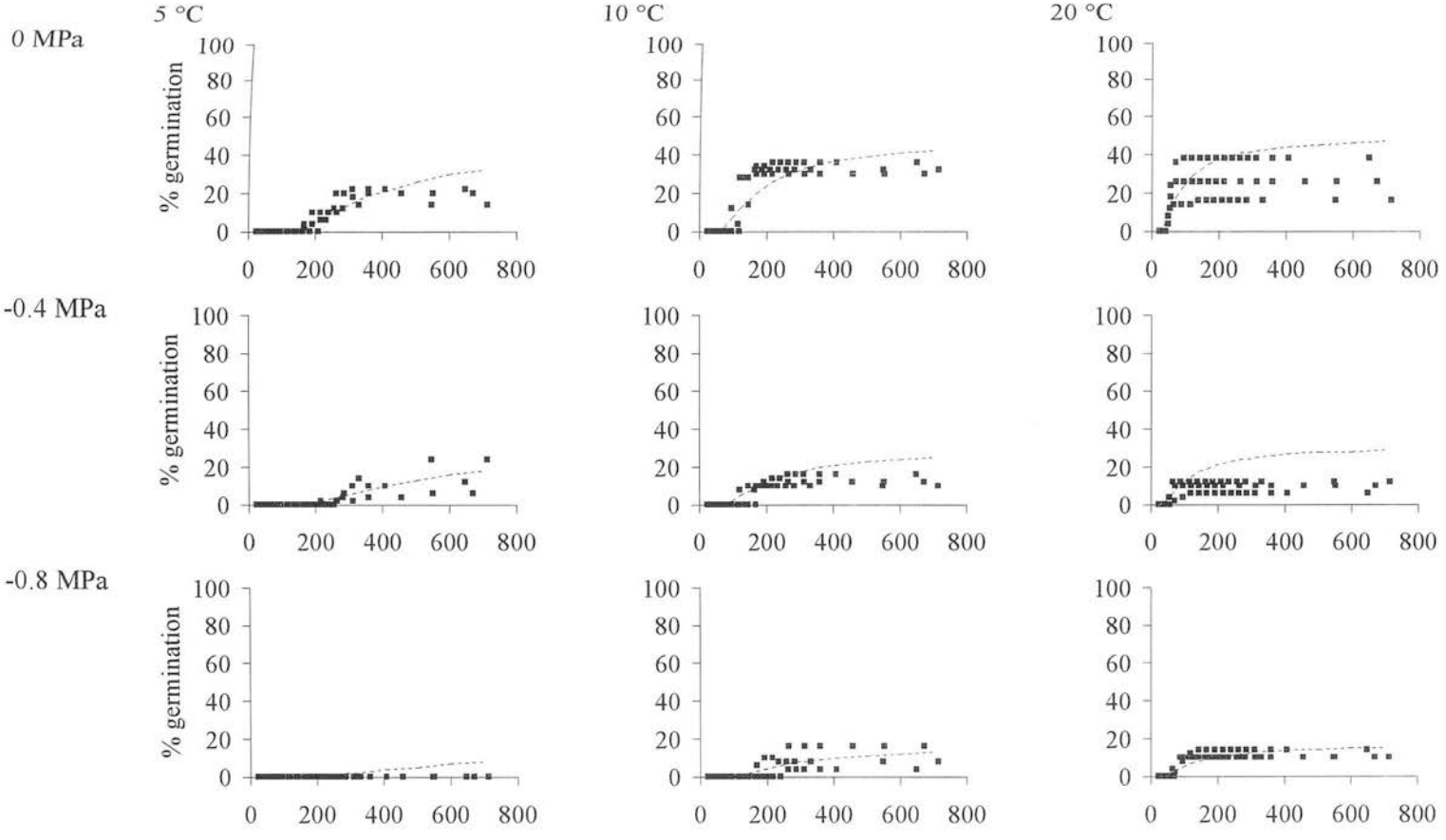


Figure 6.6: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Leicestershire population of *Stellaria media* at sub-optimal temperatures without red light at initiation. $r^2 = 0.51$.
n = 3.

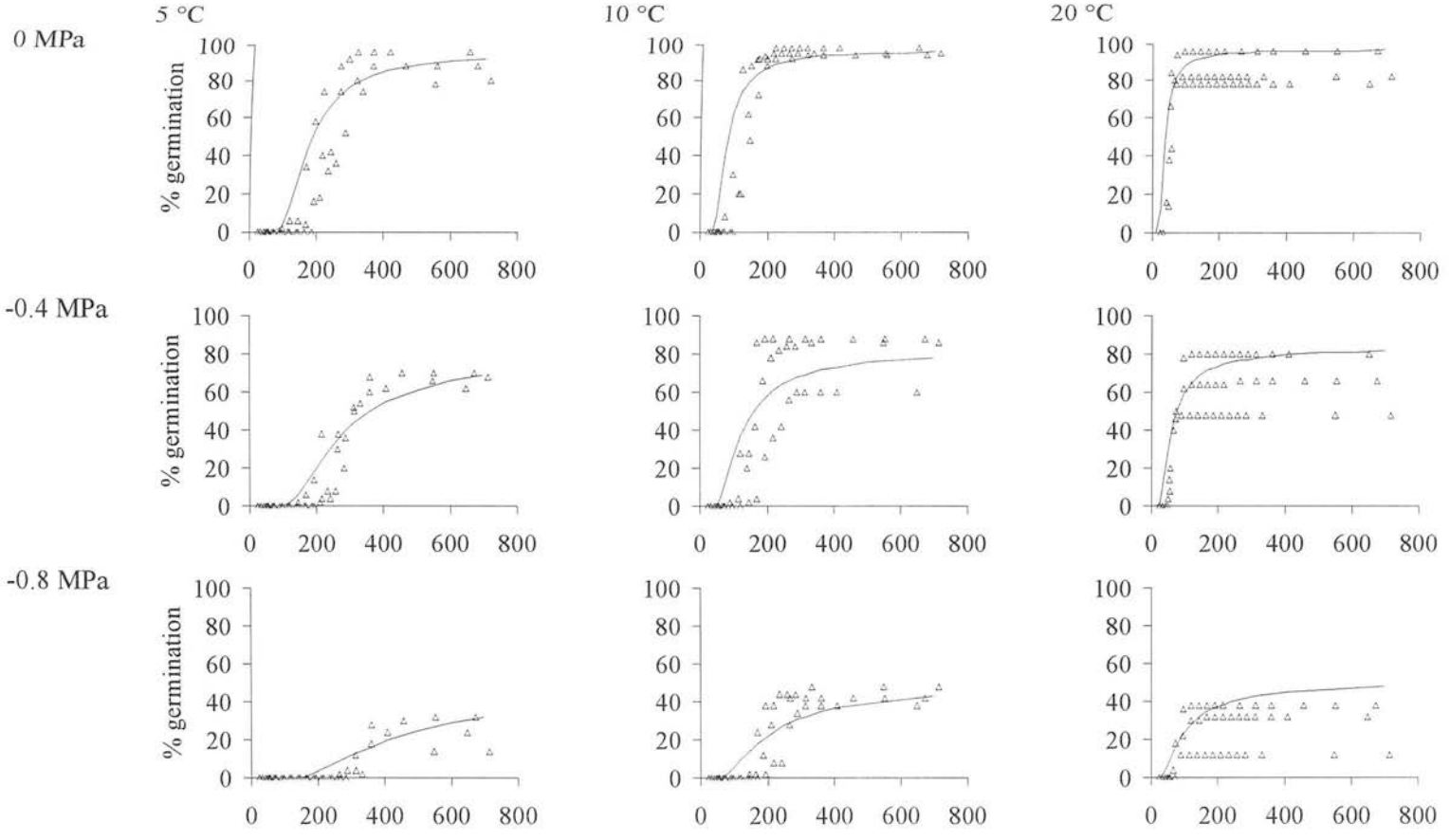


Figure 6.7: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Perthshire population of *Stellaria media* at sub-optimal temperatures with red light at initiation. $r^2 = 0.64$.
n = 3.

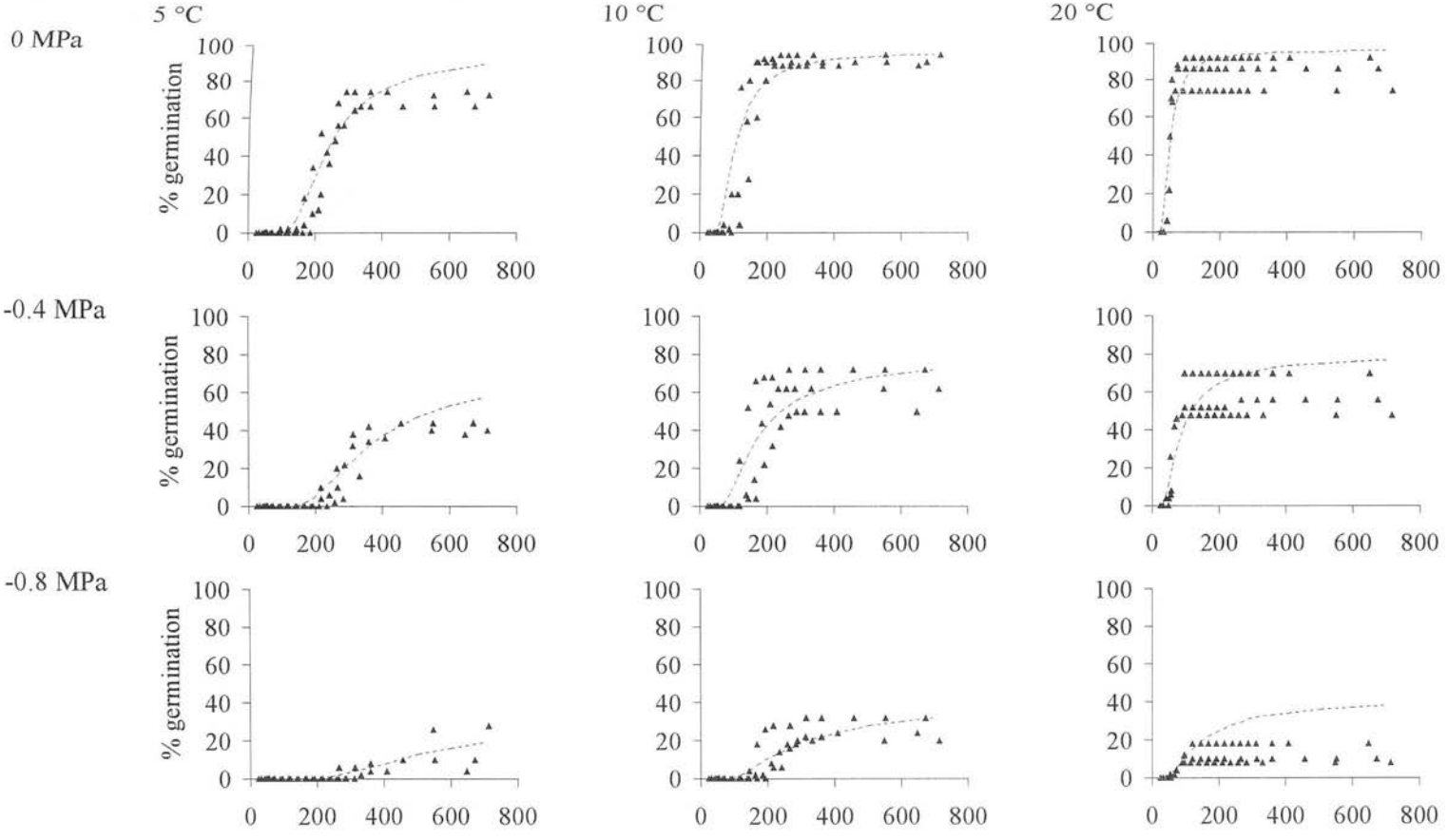


Figure 6.8: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Perthshire population of *Stellaria media* at sub-optimal temperatures without red light at initiation. $r^2 = 0.70$.
n = 3.

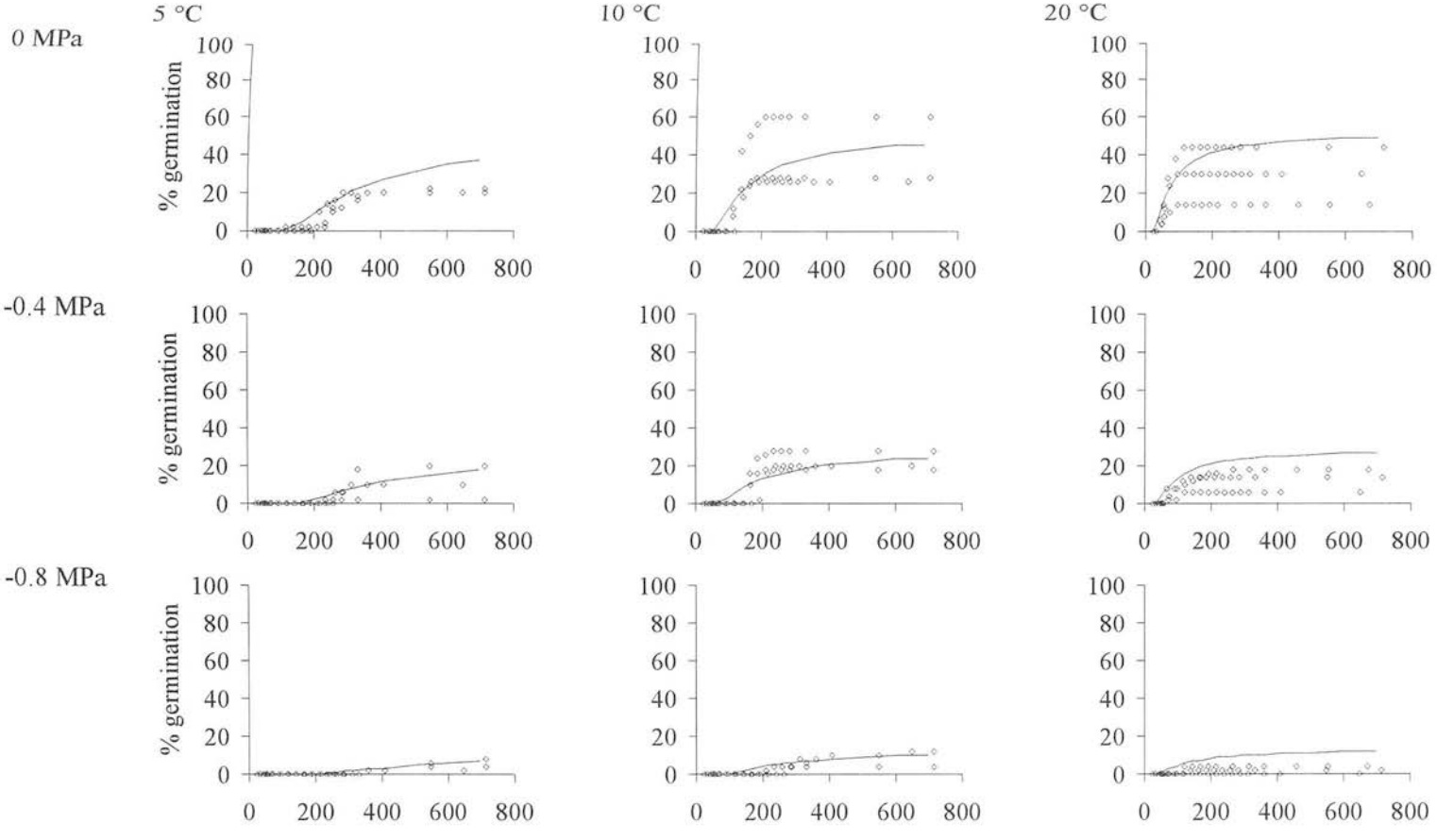


Figure 6.9: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Caithness population of *Stellaria media* at sub-optimal temperatures with red light at initiation. $r^2 = 0.54$.
n = 3.

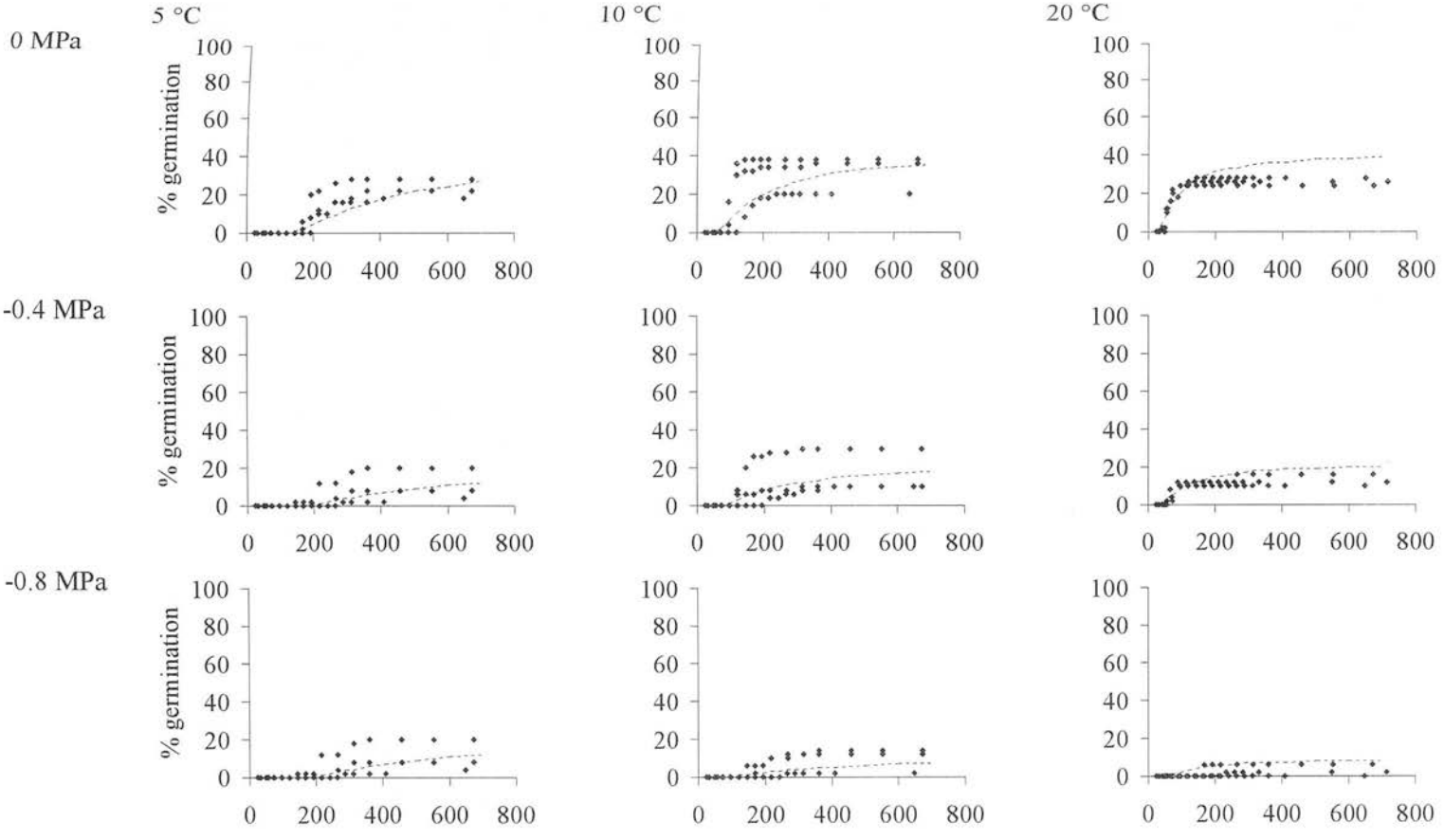


Figure 6.10: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Caithness population of *Stellaria media* at sub-optimal temperatures without red light at initiation. $r^2 = 0.60$.
n = 3.

Figure 6.5 to Figure 6.10 show that in general the start of germination tends to be reasonably well fitted, but the speed of germination is poorly fitted. In general the fits of the hydrothermal model overpredicted final percentage germination and this was largely through failure to asymptote. Model fits were best for the Perthshire population (r^2 = 0.64 in light, 0.70 in dark) and this was associated with high levels of germination.

6.5. Discussion

Three contrasting populations were used in this investigation of the effects of temperature, water potential and light on the germination of *Stellaria media*. The need to consider population differences and treat generalisations about species behaviour with caution has been illustrated by a number of authors (e.g. Andersson & Milberg, 1998, Baskin & Baskin, 1998) especially for widespread species with asexual reproduction. *S. media* is a widespread species with individuals that produce hermaphrodite flowers and frequently self pollinate (Sobey, 1981). Van der Vegte (1978) described marked differences between two populations of *S. media* from a single field in the Netherlands and Chapter 2 described marked differences in germination and seedling growth between 25 UK populations of *S. media*. The results of these experiments further illustrate the need to consider population differences prior to generalising about *S. media* germination behaviour.

Differences in population germination behaviour are a consequence of genetic differences between populations, differences in the environmental conditions in which the seed were produced (maternal effects) and stored, and the interaction between these genetic and environmental effects. The seeds used in these experiments were produced in a common environment with the aim of minimising the environmental component of variation, allowing differences between populations to be largely ascribed to genetic differences. The use of even-aged seeds produced in a common environment contrasts with Grundy (1997) who examined germination in relation to temperature and water potential for seven seed lots, varying in age from freshly harvested to five year old seed and in the environmental conditions in which the seed were produced.

Given that the three even-aged seed populations were produced in a common environment, it is apparent that, following the definition of dormancy by Vleeshouwers (1997), the populations differ in dormancy and that this difference is likely to have a largely genetic component. The Perthshire population exhibited minimal dormancy (germinated to a high

percentage across the range of constant temperatures) and the Caithness and Leicestershire populations exhibited higher levels of dormancy. Despite these differences, there were general effects of temperature and water potential on the relative number of seeds germinating and germination rate.

6.5.1. Seed germination and temperature

In common with Grundy (1997), the germination percentage tended to be maximised at temperatures between 10 and 20 °C and decreased at 30 °C. However, the large seeded Caithness population was an exception to this pattern, with the highest germination percentages recorded at 30 °C. This was associated with protracted germination and in some cases a pronounced pause and resumption of germination, resulting in two distinct phases. This would suggest that there is marked variability in this seed population and it would be interesting to determine whether this was a consequence of greater out-crossing. It is difficult to understand why a population that has evolved in Caithness germinates to a high percentage at 30 °C. Given that high temperatures and drought conditions are rarely recorded in northerly Caithness, it is unlikely that this population will have been under the same selection pressures as more southerly populations, where high temperatures are inevitably associated with limited water availability. It is interesting to note the general level of germination at 30 °C, as previous authors have reported that germination does not occur at temperatures greater than 30 °C (Roberts & Lockett, 1975; Fitter & Peat, 1994).

Temperature had an effect on the timing of germination, which decreased as temperature increased from 5 to 20 °C. This was to be expected as the reactions and processes required for germination are likely to happen at a faster rate at higher temperatures.

6.5.2. Seed germination and water potential

Germination percentage decreased as water potential decreased, a result previously reported by Grundy (1997),. Grundy (1997) recorded further reductions in germination percentage as water potential decreased from –0.8 to –1.4 MPa. Reduced water potential also resulted in reduced germination rate.

Significant interactions between temperature and water potential were recorded, with the reduction in germination percentage with decreased water potential being less marked at

10 °C when compared with 5 and 20 °C. The reduction in germination percentage with reduced water potential at 30 °C varied markedly between populations, with high levels of germination maintained at –0.4 and –0.8 MPa for the Caithness population. Again this might reflect the unlikely exposure of this population to conditions of such high temperatures and low water availability.

6.5.3. Seed germination and light

The populations were also shown to differ markedly in their response to light. This was informative as light has variously been reported to be an absolute requirement for *S. media* germination at constant temperatures (Roberts & Lockett, 1975); to significantly increase the proportion of buried seeds that germinate (Wesson & Wareing, 1969a & b; Baskin & Baskin, 1979; Kryger Jensen, 1995) and to have no effect whatsoever (Grime *et al.*, 1981; Grime, Hodgson & Hunt, 1988). Red light at initiation was shown to significantly increase the germination percentage of the Leicestershire population across all treatments. Germination of the Perthshire population also significantly increased, but less markedly, suggesting that a proportion of these seeds are either light insensitive or sensitive to the very low levels of light given by the green safe light.. Differences for the Caithness population were restricted to a sub-set of conditions, at lower water potentials and at 5 or 10 °C. Consequently, it may be that the confusion of previous reports is the result of differences between distinct populations. It is not clear why the Leicestershire population had a pronounced requirement for red light to initiate germination, but it could be argued that under greater intensity of farming (e.g. continuous cropping as commonly occurs in southern Britain), an adaptation that limits seed germination to periods following cultivation would be selective.

6.5.4. Seed germination and the hydrothermal model

Gummerson (1986) suggested that seed germination responses according to both temperature and water potential could be combined into a single expression on a hydrothermal time basis. As described previously, this approach has been applied, with some success, to crop plants. Grundy (1997) outlined the problem of applying this analysis to predict weed germination time courses. The main problems are that weed seeds tend to have a more variable germination response than crop plants. In predicting field germination, this variation in weed seed germination may be a consequence of seed age, seed history and the genetic

structure of the seed population. In this analysis attempts have been made to eliminate the problem of seed age and seed history.

Grundy (1997) attempted to apply the hydrothermal time model to a three years old seed lot of *S. media*, selected specifically on account of low dormancy. A base temperature (T_b) of 4.7 °C was identified by linear regression of the germination rate ($1/t(50)$) against test temperatures of 10, 15 and 20 °C. It should be noted that it was not clear whether germination fraction was defined according to the total number of seeds or the total number of germinable seeds in the experiment, although selection of a population with low levels of dormancy minimised the impact of this distinction. Grundy (1997) excluded germination rates at 5 °C because of non-linearity and this may be one reason for the higher base temperature identified. However in these experiments there was no indication of similar non-linearity at 5 °C, although there was marked variation in the germination rates at 20 °C (Figure 6.4). It is possible that this variation might be explained by the speed of germination at 20 °C, which was incompatible with the recording interval. More frequent recording of initial germination might have reduced this variation, especially for the time to 10 and 20 % germination, but this would have meant more frequent removal of the seeds from the test temperature. However, if it is accepted that there is a linear relationship between germination rate and temperature, it is clear from Table 6.5 that the probit method of estimating base temperature, T_b, was preferable (produced a single value) compared with estimating according to an arbitrarily selected germination fraction.

Using repeated probit regression, base temperatures between 0.3 and 2.1 °C were estimated in light and 0.8 and 1.4 °C in darkness, according to population (Table 6.5). There is only a relatively small variation in base temperatures between different populations and different light conditions. Base temperatures tended to be higher in light, except for the Leicestershire population. There is no reason to suggest that this might be significant, although the Leicestershire population was the population that exhibited the greatest response to light. In both light and dark treatments, the highest base temperatures were estimated for the Perthshire population, though again, there is no reason to suggest that this might be significant.

Grundy (1997) presented the rate of germination in thermal time against water potential and demonstrated that the points for each germination fraction fell on a series of approximately parallel lines (data from 10, 15 and 20 °C). Although this is a necessary step in further

developing the hydrothermal time model (illustrating variation in base water potential and allowing estimation of the hydrothermal time constant from the gradient of the parallel lines), these parameters were not presented. Moreover, the hydrothermal time model was not then compared with the actual data and discussed. From examination of the graph of germination rate in thermal time versus water potential it would appear that the hydrothermal time constant (θ_{HT}) would be estimated as 100 MPa °C day^{-1} or 2400 MPa °C h^{-1}. It was not possible to estimate ψ_{b50} or $\sigma_{\psi b}$. It is clear that this value for the hydrothermal time constant varies markedly from the values presented in Table 6.6. The reason for this difference is not clear because, although the base temperature identified in Grundy (1997) was higher, higher base temperatures would tend to reduce the hydrothermal time constant. In Table 6.6 the values for the hydrothermal time constant vary from 595 to 1395 MPa °C h^{-1} in light and 735 to 1085 MPa °C h^{-1} in darkness, according to population. Differences between populations were consistent with the least dormant population, the Perthshire population, having the lowest hydrothermal time constant.

The usefulness of the hydrothermal time model is demonstrated by the fit to the actual data, as shown in Figure 6.5 to Figure 6.10. Overall the onset of germination tended to be well fitted, except for the Perthshire population (all conditions) and the Caithness population with light and at temperatures greater than 10 °C. In both cases the onset of germination was underestimated. Overall, there was a clear tendency for the hydrothermal time model to underestimate the speed of germination, though this was less obvious at higher temperatures. Additionally, final germination percentage tended to be overestimated, with the exception of the Caithness population at lower temperatures where actual germination was higher than that fitted. The most serious overestimation of the final germination percentage was for the Leicestershire population in light at both 5 and 20 °C. From this discussion , it is clear that there were a number of failings in the ability of the hydrothermal model to fit the data and possible explanations are given below. However it was reassuring to note that general trends and differences between populations and in final germination percentage with light at initiation compared to darkness, are reflected in the fitted hydrothermal model.

There are two main problems with the hydrothermal time model (as defined by Gummerson, 1986). Firstly, no statistical methods have previously been used to calculate confidence intervals for either the model output or parameter estimates. In Chapter 11 this problem is addressed by formulating a stochastic model of the germination process. However within the existing model framework, bootstrap estimation was applied to the base temperatures.

This resulted in the confidence intervals given in Table 6.5. Although, in principle, it is feasible to extend this method to estimate confidence intervals for other parameters and also for model output, this would be computationally intensive.

Secondly, the hydrothermal time model is flawed in application to seed populations with dormancy as the model structure requires that final percentage germination is 100 percent. Consequently, when final percentage germination is less than 100 percent, the hydrothermal time model compensates by reaching the asymptote very slowly (see Figure 6.5). This failure to asymptote probably leads to other problems such as poor estimation of the speed of germination. It is therefore clear that a parameter to estimate final percentage germination should be included in the model.

Additionally, the hydrothermal time model can be criticised for a general lack of consistency, for example the difference in the treatment of thermal time in equation (6.5) and hydrothermal time in equation (6.7). Moreover it is not clear how to extend the model to account for other environmental factors, such as light or nitrogen. Finally, it should be noted that the linear nature of the model (on a probit scale) means that sub- and supra-optimal temperatures must be treated separately, which in this case restricts analysis to sub-optimal temperatures. Given these criticisms, it was decided to adopt an alternative modelling framework and this is described in Chapter 11.

6.6. Conclusions

Final percentage germination and the timing of germination differed significantly between populations of *S. media* according to temperature, water potential and light conditions at initiation. There were also a large number of significant interactions between environmental factors. In general, final percentage germination was maximised at 10 °C in water and following an initial exposure to red light. Lower water potentials tended to decrease final percentage germination. The time to the onset of germination tended to decrease with increasing temperature up to 20 °C and increased at higher temperatures. It also increased with lower water potentials. Germination also tended to be most synchronous at 20 °C and in water.

There is evidence that differences between populations may be related to differences in the physical environment or management practices between localities. The most northerly

population was recorded with maximum final percentage germination at 30 °C and germination also tended to be maintained at lower water potentials. It was suggested that this may be a consequence of evolution in the absence of selection pressures to limit germination in conditions of high temperature and/or limiting water availability. It was also noted that the population with the greatest sensitivity to light derived from the most southerly population, from an area that traditionally associated with intensive cultivation.

Application of the hydrothermal model was problematic in that, although the onset of germination was reasonably well determined, germination speed tended to be underestimated and final percentage germination was overestimated. This was largely associated with a failure to asymptote. Failure to asymptote is inherent in the structure of the hydrothermal time model and this together with difficulties in estimating confidence intervals for model output and parameter values undermine the utility of this method.

Chapter 7. The effect of temperature and water potential on seed germination in contrasting populations of *Galium aparine*.

7.1. Summary

An experiment was conducted to investigate the effect of temperature and water potential on the germination time courses of three contrasting populations of *Galium aparine*. The data were then used to assess the utility of using the hydrothermal time model (Gummerson, 1986) to describe germination patterns for *G. aparine*.

Timing and extent of germination for the three contrasting populations of *Galium aparine* were affected by temperature and water potential. Final percentage germination was maximised at temperatures of 5 and 10 °C and decreased at higher temperatures. Germination was also more protracted at 20 °C. Water potentials of –0.4 MPa reduced final percentage germination only at higher temperatures (> 15 °C), but water potentials of –0.8 MPa reduced final percentage germination at all temperatures. There was an indication that final percentage germination of the smaller seeded population was disproportionally reduced at -0.8 MPa and higher temperatures. Lower water potentials also increased the time to germination.

Application of the hydrothermal time model was restricted to germination at temperatures less than 10 °C. Failure to fit a base temperature to one of the three populations (by probit regression analysis) also limited the utility of the model. Despite near complete germination in optimal conditions for the two *G. aparine* populations to which the model was fitted, the model consistently overestimated germination at low water potentials and for one population the estimated start of germination was too early. This failure to asymptote correctly together with the lack of confidence intervals, questions the utility of this model for predicting weed population germination time courses.

7.2. Introduction

Temperature has long been recognised as an important factor in determining the timing and duration of flushes of weed seedlings (Hegarty, 1973, Bouwmeester & Karssen, 1992). Soil

water potential has also been recognised as an important factor (Roberts, 1984), but there has generally been less work to describe the effect of water potential and the interaction between temperature and water potential on patterns of seed germination. Investigations concerned with the effect of temperature and water potential on the extent and timing of germination have been restricted to *Stellaria media* (Grundy, 1997 and Chapter 6) and *Chenopodium album, Amaranthus retroflexus* and *Echinochloa crus-galli* (Martinez-Ghersa, Satorre & Ghersa, 1997). Other investigations have been concerned with the effect of temperature and water potential on either the timing of germination (Weaver, Tan & Brain, 1988) or final percentage germination only (van der Weide, 1993).

With the exception of Grundy (1997) and Chapter 6 of this thesis, it should be noted that investigations to date have also tended to be restricted to single population studies. In order to model the processes of seed germination, data on the extent and timing of seed germination need to be combined, together with realistic definition of the extent of variation between populations. Chapter 6 examined the use of the hydrothermal time model defined by Gummerson (1986) to describe the timing and extent of *S. media* germination for three contrasting populations. It is the aim of this Chapter to gather similar data for contrasting populations of *Galium aparine*. This will allow assessment of the effect of temperature and water potential on patterns of *G. aparine* seed germination and further consideration of the utility of the hydrothermal time model.

Current information concerned with the effect of temperature and water potential on *G. aparine* germination is restricted to a single study by van der Weide (1993). This study compared *G. aparine* seedling emergence at 8 and 14 °C for seeds from an arable and hedgerow population that were buried at 2 cm depth in a clay soil maintained at a range of five gravimetric soil moisture contents between 9.3 and 28.9 %. For a clay soil this may approximate to a range in soil water potentials from 0 to -1.5 MPa (Rundel & Jarrell, 1989). Van der Weide (1993) concluded from these experiments that under optimal temperature conditions (8 °C) soil moisture content had no effect on final percentage germination (emergence) of *G. aparine*. Under sub-optimal temperature conditions (14 °C), final percentage germination (emergence) was reduced at soil moisture contents from 18 to 9.3 % (-0.6 to –1.5 MPa (Rundel & Jarrell, 1989)). No significant differences in response were recorded according to population.

Regarding the effect of soil water content alone, Ferris (1988) investigated patterns of seedling emergence for an unpaired field and hedgerow population of *G. aparine* under greenhouse conditions with either daily or alternate-day watering. Ferris (1988) recorded lower seedling emergence with less frequent watering, especially for the field population and it was suggested that this might reflect greater sensitivity of field populations to conditions of low water potential.

From this review of the effect of temperature and water potential on *G. aparine* germination, it is clear that there is insufficient information to adequately model the process. It is also clear that there could be substantial variation in response according to population and the work of van der Weide (1993) suggests that overall germination of *G. aparine* might be less sensitive to the effect of low water potentials than that observed in Chapter 6 for *S. media*.

The effect of light was not included as an additional factor in this series of experiments as it is well described in the literature. There is general consensus that *G. aparine* germination is maximised at low levels of light (Grime *et al.*, 1981; Bliss & Smith, 1985) and that high levels of light (Bliss & Smith, 1985; Malik & van den Born, 1988) and far red wavelengths (van der Weide, 1993) are inhibitory.

7.3. Methods

7.3.1. Seed used

Mature seed was harvested in August 1998 from three populations of *G. aparine* (as identified in Chapter 3). The seed was harvested from plants grown in an outdoor cage. The plants were grown in three blocks, with populations grouped together within the blocks and separated by greater than 2 metres to limit cross-pollination. Seeds were stored dry in an incubator maintained at 10 °C prior to the experiment.

7.3.2. Experimental design and treatments

In June 1999, experiments were established to assess the germination time course of the three populations in all combinations of five constant temperatures and three water potentials. All seeds were exposed to red light for 15 minutes at initiation (and subsequently to intermittent very low levels of green light). The five test temperatures were 5, 10, 15, 20 and 30 °C and the three water potentials were 0, -0.4 and -0.8 MPa.

For each germination test, 30 seeds were placed on a double layer of filter paper (Whatman no. 181) in a 9 cm plastic Petri-dish. There were three replicates for each treatment, established at weekly time intervals to counter the lack of replication in incubators. Solutions differing in water potential were prepared using polyethylene glycol 8000 (PEG), with different concentrations of PEG used for each temperature and water potential combination as described by Michel (1983). The PEG solutions were prepared in bulk. Under a green safe light, each Petri-dish received 10 ml of the appropriate PEG solution or distilled water and was then sealed in a self-seal clear polythene bag, to minimise changes in water potential due to evaporation. The green safe light was given by two Osram L 36W/30 fluorescent tubes with diffusers covered with two layers of dark yellow-green Cinemoid filter (Lee filters #90), giving a photosynthetically active photon flux density (PPFD) of 0.51 $\pm$ 0.19 μmol m^{-2} s^{-1}. The seeds were then given red light for 15 minutes beneath two Osram L 36W/30 fluorescent tubes with diffusers covered with two layers of bright red Cinemoid filter (Lee filters #26), giving a PPFD of 3.84 $\pm$ 0.71 μmol m^{-2} s^{-1} (PPFD recorded by quantum sensor and a Campbell CR10 datalogger). These Petri-dishes were then randomly placed (under the green safe light) in sealed cardboard boxes wrapped in black polythene and lined with bubble wrap, with a separate box for each temperature. The boxes were then removed to unlit incubators (Gallenkamp/Cryotechnics, cooled) maintained at the defined constant temperatures of 5, 10, 15, 20 and 30 °C ($\pm$ 2 °C). Germination was assessed under the green safe light, daily for the first 15 days and then at less frequent intervals until germination ceased. Seed germination was defined as radicle emergence to greater than 1 mm and germinated seeds were removed from Petri-dishes when recorded.

7.3.3. Initial data analysis

To summarise the data, individual Gompertz curves were fitted to cumulated seed germination counts for each Petri-dish. The Gompertz curve was defined as in Chapter 6.

Statistical analysis of population and treatment effects used analysis of variance (Genstat 5) to compare the fitted Gompertz parameters and derived estimates of mean percentage germination and time to 50% germination. Data sets that were poorly fitted by the Gompertz curve ($r^2 < 0.85$), where parameter μ was negative or where no germination was recorded were excluded from the analysis.

7.3.4. Calculation of base temperatures and application of the hydrothermal model

Application of the hydrothermal model used the methods defined in Chapter 6. Probit analysis, bootstrap estimation and fitting of the hydrothermal time models used purpose written routines (Dr. Glenn Marion) within the statistical package R (http://www.ci.tuwien.ac.at/R/). It should be noted that all available data were used to fit the models and no independent model validation is presented. As such r^2 statistics describe model goodness of fit and are likely biased estimates of the models predictive ability.

7.4. Results

The Gompertz distribution was fitted to cumulated counts of germinated seeds for each Petri-dish where seeds germinated. No germination was recorded in 5 Petri-dishes, including all three replicates of the Northumberland population at 20 °C in -0.8 MPa PEG. The percentage variance accounted for by the fitted Gompertz curves exceeded 85 % in all except one of the remaining 103 data sets. This one data set and five additional data sets where parameter μ (the mean time to germination) was recorded as a negative number were excluded from further analysis. These included all three replicates of the Northern Ireland population at 20 °C in distilled water where germination was observed to occur in distinct phases or steps. No germination was recorded in any treatment at 30 °C.

7.4.1. Percentage germination

Table 7.1 shows the mean percentage germination of *G. aparine* seeds after 70 days according to population and treatment. For all treatments, no substantial germination was recorded after 60 days. Table 7.1 shows that for each population, the percentage of seeds germinating decreased as water potential decreased. Table 7.4 (summary of analysis of variance) shows that there were significant differences in final percentage germination ($\alpha + \gamma$) between populations and according to temperature. Final percentage germination was significantly lower for the North Yorkshire population and tended to be lower at both 15 and 20 °C for all populations. The reduction in final percentage germination with decreasing water potential was also particularly pronounced at 15 and 20 °C.

7.4.2. Germination time

Table 7.2 shows the mean value of the Gompertz parameter μ (which measures the time to the point of inflexion) according to population and treatment. Table 7.2 and Table 7.4 (summary of analysis of variance) show that for each population, the time to the point of inflexion of the Gompertz curve significantly increased as water potential decreased. The time to inflexion also interacted with temperature, increasing as temperatures decreased from 15 to 5 °C. Generally there were no consistent differences between the time to inflexion at 15 compared with 20 °C, except at –0.8 MPa, where the time to inflexion was consistently longer at 20 °C. Considering all treatments (except those at 20 °C) time to inflexion tended to be shortest for the Northumberland population and longest for the Northern Ireland population.

7.4.3. Synchronicity of germination

Table 7.3 shows the mean value of Gompertz parameter β (which measures the extent of synchronicity in seed germination) according to population and treatment. Table 7.4 summarises the significance of these differences. Table 7.3 shows that for each population and water potential treatment germination tended to be least synchronous at 5 °C, although there were notable exceptions. Table 7.3 also shows that for all three populations, there was a significant trend to lower synchronicity of germination in the –0.4 MPa water potential treatment. There was a significant interaction between test temperature and water potential, with markedly higher synchronicity recorded for –0.8 MPa at 20 °C. However it should be noted that this might reflect the low levels of germination recorded in this these treatments.

7.4.4. Overall patterns of germination

Figure 7.1, Figure 7.2 and Figure 7.3summarise the general patterns of seed germination according to population, temperature and water potential using Gompertz fitted curves.

Population	Water potential	Temperature 5 °C	10 °C	15 °C	20 °C
North Yorkshire	0 MPa	75.64	71.96	65.14	70.10
	-0.4 MPa	73.20	72.62	25.95	35.55
	-0.8 MPa	32.45	29.39	9.70	8.15
Northumberland	0 MPa	98.80	97.26	98.40	92.61
	-0.4 MPa	100.23	97.97	20.35	39.15
	-0.8 MPa	25.96	21.46	7.58	0
Northern Ireland	0 MPa	95.68	98.50	61.96	*
	-0.4 MPa	94.32	94.23	41.94	47.99
	-0.8 MPa	18.40	41.41	17.10	3.08

Table 7.1: Mean percentage germination of *Galium aparine* seeds according to population, temperature and water potential following exposure to red light at initiation. Results are as estimated by summing Gompertz parameters ($\alpha + \gamma$) and include zero values for samples where no germination was recorded.
No germination was recorded at 30 °C. * = Failure to fit Gompertz curve.

Population	Water potential	Temperature 5 °C	10 °C	15 °C	20 °C
North Yorkshire	0 MPa	249.52	160.74	140.44	163.07
	-0.4 MPa	438.75	296.07	224.89	232.06
	-0.8 MPa	708.86	509.22	353.98	432.13
Northumberland	0 MPa	251.75	145.05	209.89	207.68
	-0.4 MPa	426.62	333.68	161.95	368.31
	-0.8 MPa	550.53	311.17	196.97	*
Northern Ireland	0 MPa	284.42	161.45	173.76	*
	-0.4 MPa	491.70	351.05	267.03	238.38
	-0.8 MPa	768.71	620.43	354.60	392.57

Table 7.2: Mean Gompertz parameter μ (time to point of inflexion, hours) for *Galium aparine* seeds according to population, temperature and water potential following exposure to red light at initiation.
No germination was recorded at 30 °C. * = Failure to fit Gompertz curve.

Population	Water potential	Temperature 5 °C	10 °C	15 °C	20 °C
North Yorkshire	0 MPa	0.0185	0.0295	0.0151	0.0088
	-0.4 MPa	0.0150	0.0145	0.0082	0.0199
	-0.8 MPa	0.0046	0.0117	0.0306	0.0266
Northumberland	0 MPa	0.0184	0.0271	0.0120	0.0084
	-0.4 MPa	0.0083	0.0071	0.0263	0.0039
	-0.8 MPa	0.0055	0.0189	0.0237	*
Northern Ireland	0 MPa	0.0193	0.0284	0.0138	*
	-0.4 MPa	0.0073	0.0079	0.0070	0.0117
	-0.8 MPa	0.0062	0.0042	0.0061	0.0285

Table 7.3: Mean Gompertz parameter β (synchronicity in seed germination, hour^{-1}) for *Galium aparine* seeds according to population, temperature and water potential following exposure to red light at initiation.
No germination was recorded at 30 °C. * = Failure to fit Gompertz curve.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	2	2.18	-	2.49	-	17.58	***
Temperature	3	3.10	*	27.68	***	108.91	***
Water potential	2	7.87	***	69.22	***	575.66	***
Population x temperature	6	2.95	*	1.67	-	2.03	-
Population x water potential	4	2.12	-	4.93	**	14.32	***
Temperature x water potential	6	9.96	***	3.03	*	23.28	***
Population x temperature x water potential	10	2.54	*	0.41	-	4.45	***
Residual	61						

(11 missing values)

Table 7.4: Summary of the analysis of variance for the differences between *Galium aparine* populations for fitted Gompertz parameters according to temperature and water potential.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$

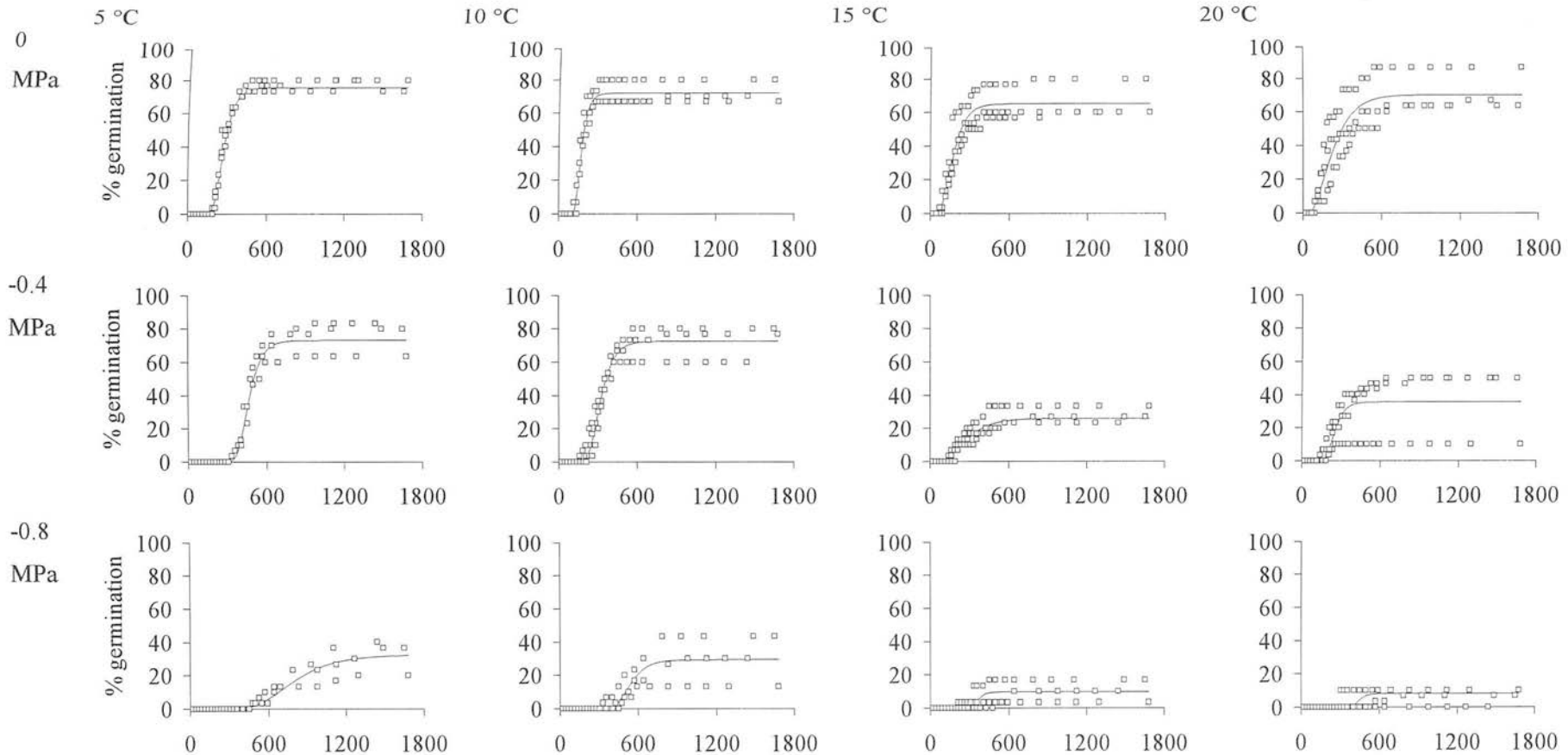


Figure 7.1: Fitted Gompertz curves for germination time courses at 5, 10, 15 and 20 °C and at water potentials of 0, -0.4 and –0.8 MPa for seeds of the North Yorkshire population of *Galium aparine*, following red light at initiation.
n = 3.

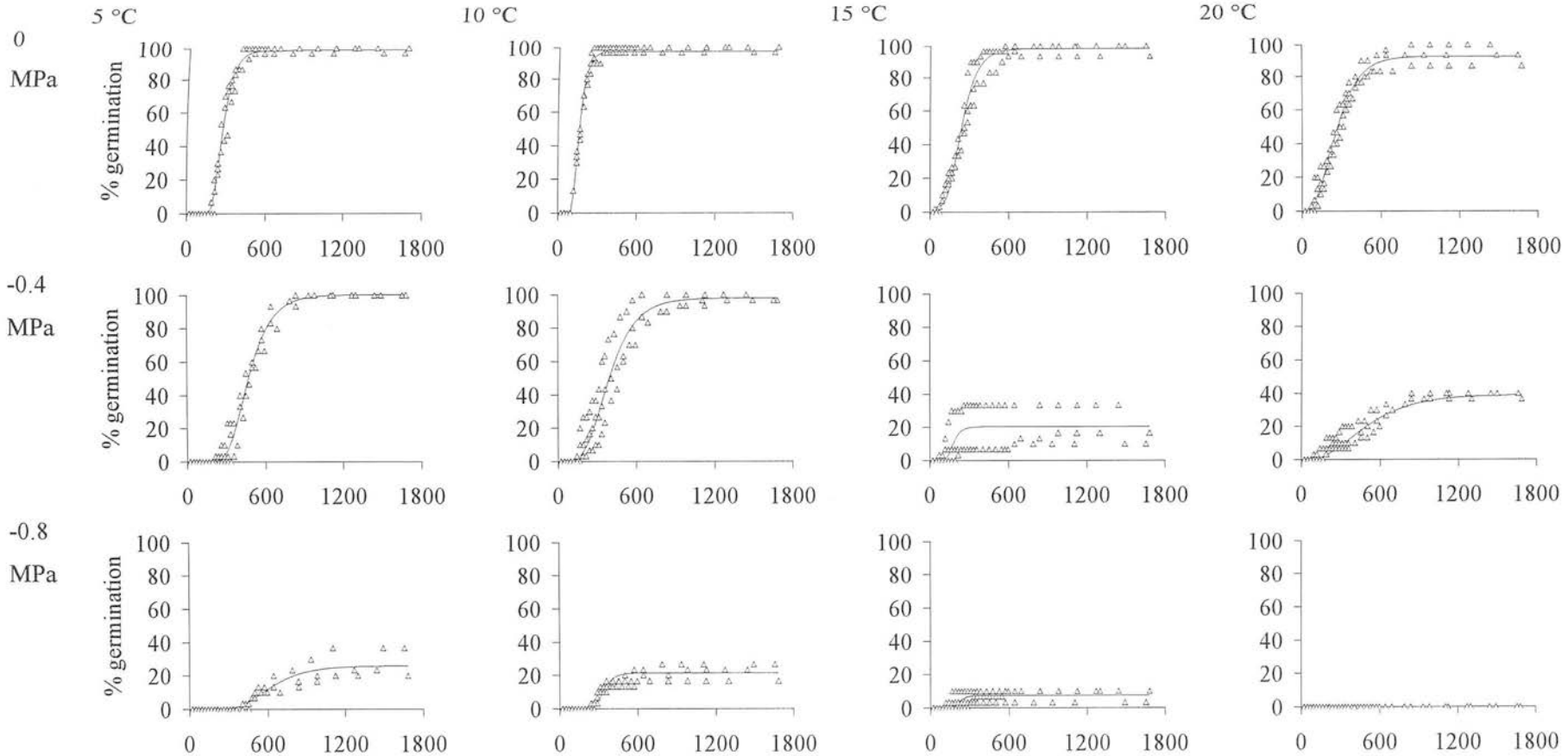


Figure 7.2: Fitted Gompertz curves for germination time courses at 5, 10, 15 and 20 °C and at water potentials of 0, -0.4 and –0.8 MPa for seeds of the Northumberland population of *Galium aparine*, following red light at initiation.
n = 3.

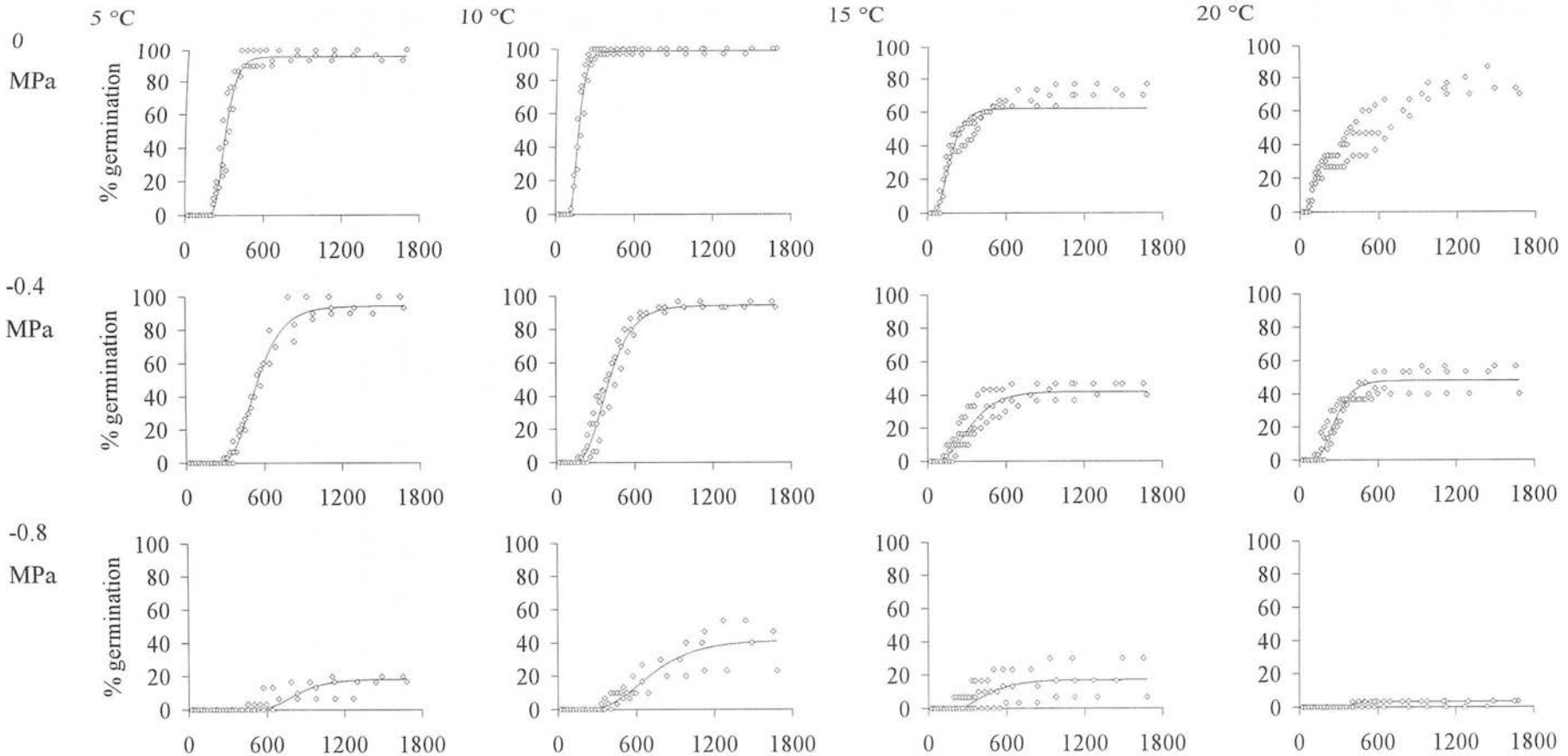


Figure 7.3: Fitted Gompertz curves for germination time courses at 5, 10, 15 and 20 °C and at water potentials of 0, -0.4 and –0.8 MPa for seeds of the Northern Ireland population of *Galium aparine*, following red light at initiation.
n = 3.

7.4.5. Germination rate and calculation of base temperatures

Base temperatures were calculated by two different methods, linear regression and repeated probit regression analysis. As detailed in Chapter 6, both methods define base temperatures by identifying a linear relationship between germination rate in water and temperature at sub-optimal temperatures. Table 7.5 details the range of base temperatures estimated by these two methods and in both cases sub-optimal temperatures were defined as 5 and 10 °C. This was because germination rate decreased at higher temperatures. It should also be noted that in common with Chapter 6, the germination fraction g was determined as a fraction of the total number of seeds in each experiment.

Linear regression of germination rates ($1/t(g)$) against temperature showed variation in T_b according to germination fraction and Table 7.5 presents the estimates from linear regression of $1/t(50)$. Figure 7.4 shows the fitted linear relationships between temperature and germination rate at 5 and 10 °C for each of the three populations in distilled water following exposure to light. It is clear that the linear relationship holds between 5 and 10 °C, but the linear relationship breaks down at higher temperatures. The estimated base temperature of the North Yorkshire population was notably lower than that for the other two populations.

Estimates for base temperature from repeated probit regression analysis were restricted to the populations from Northumberland and Northern Ireland. This was because the procedure failed to fit a base temperature for the population from North Yorkshire. In contrast to the linear regression method, the base temperature of the Northumberland population was markedly lower than that of the Northern Ireland population. Confidence intervals were calculated by bootstrap estimation (as described in Chapter 6).

Despite failure to fit a base temperature for the North Yorkshire population, the probit method was used to apply the hydrothermal time model to the other two populations. This was because this method is less arbitrary and more statistically rigorous..

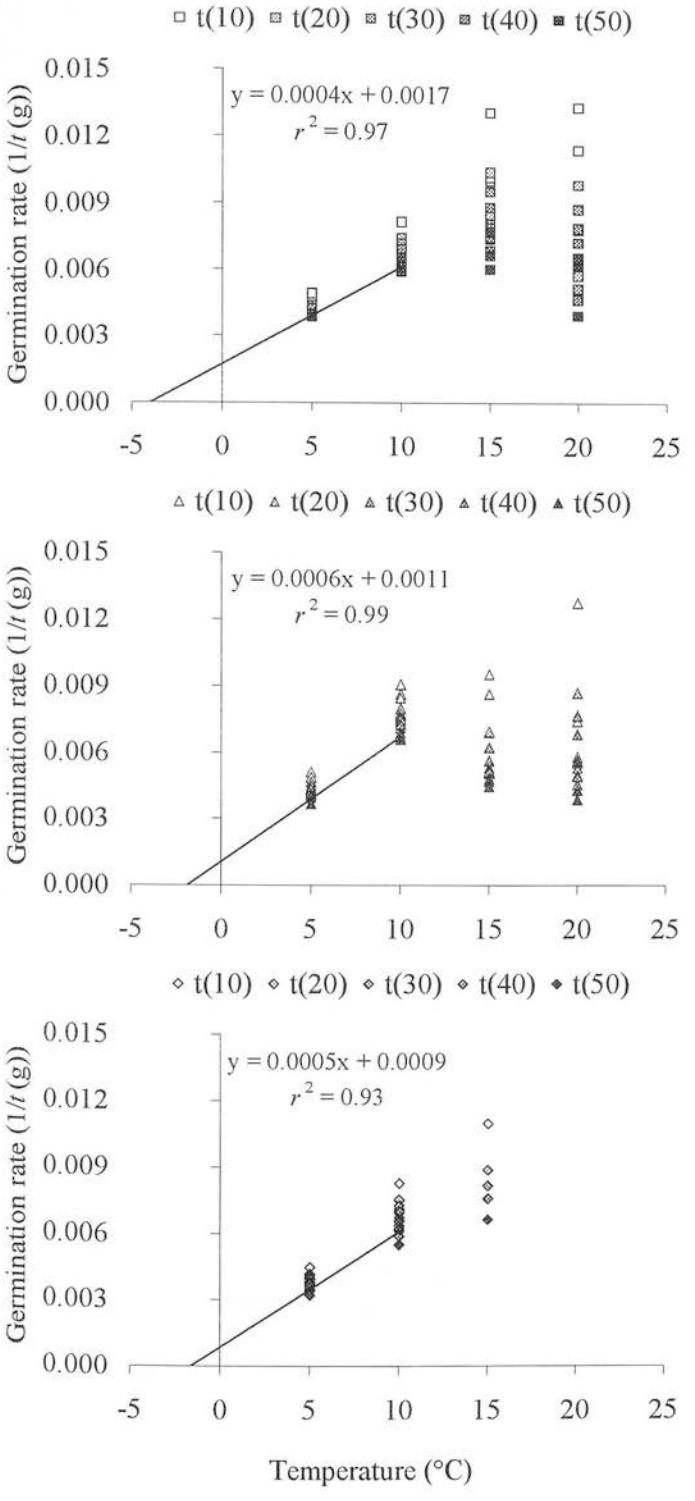


Figure 7.4: The effect of temperature on germination rate ($1/t(g)$) for different cumulative germination fractions of *G. aparine* populations . Data are restricted to seed germination in distilled water following exposure to red light on initiation. Linear regression is fitted to data at 5 and 10 °C.
n = 3.

Population	Probit	95% confidence interval.		Interpolation
North Yorkshire	*	*	*	-4.25 °C
Northumberland	-3.6 °C	-8.6 °C	-0.9 °C	-1.83 °C
Northern Ireland	0.6 °C	-5.8 °C	1.5 °C	-1.80 °C

Table 7.5: Estimated base temperatures (°C) from repeated probit regression analysis (according to equation (6.5)) or from linear regression of germination rate ($1/t(50)$) against temperature and interpolation of T_b. In each case base temperature is estimated from sub-optimal temperatures (5 & 10 °C) in water, following exposure to red light for 15 minutes on initiation. 95% confidence intervals are estimated by bootstrap resampling.

7.4.6. Application of the hydrothermal time model

Table 7.6 details the fitted parameters from the hydrothermal time model. Figure 7.5 compares the fitted germination time courses from the hydrothermal time model with experimental results for the Northumberland population at sub-optimal temperatures. Figure 7.6 compares the fitted model to seed germination for the Northern Ireland population.

Table 7.6 shows that the fitted parameters varied markedly between populations. The hydrothermal time constant of the Northumberland population was approximately double that of the Northern Ireland population, but estimates for the mean base potential and variance were similar for the two populations.

Population	T_b	θ_{HT}	ψ_b50	$\sigma_{\psi b}$
	°C	MPa °Ch	MPa	MPa
Northumberland	-3.6	1985	-2.04	0.23
Northern Ireland	0.6	1105	-1.88	0.22

Table 7.6: Estimated hydrothermal time model parameters from repeated probit regression analysis (according to Chapter 6, equation (6.7)), for *Galium aparine* populations germinated following exposure to light.

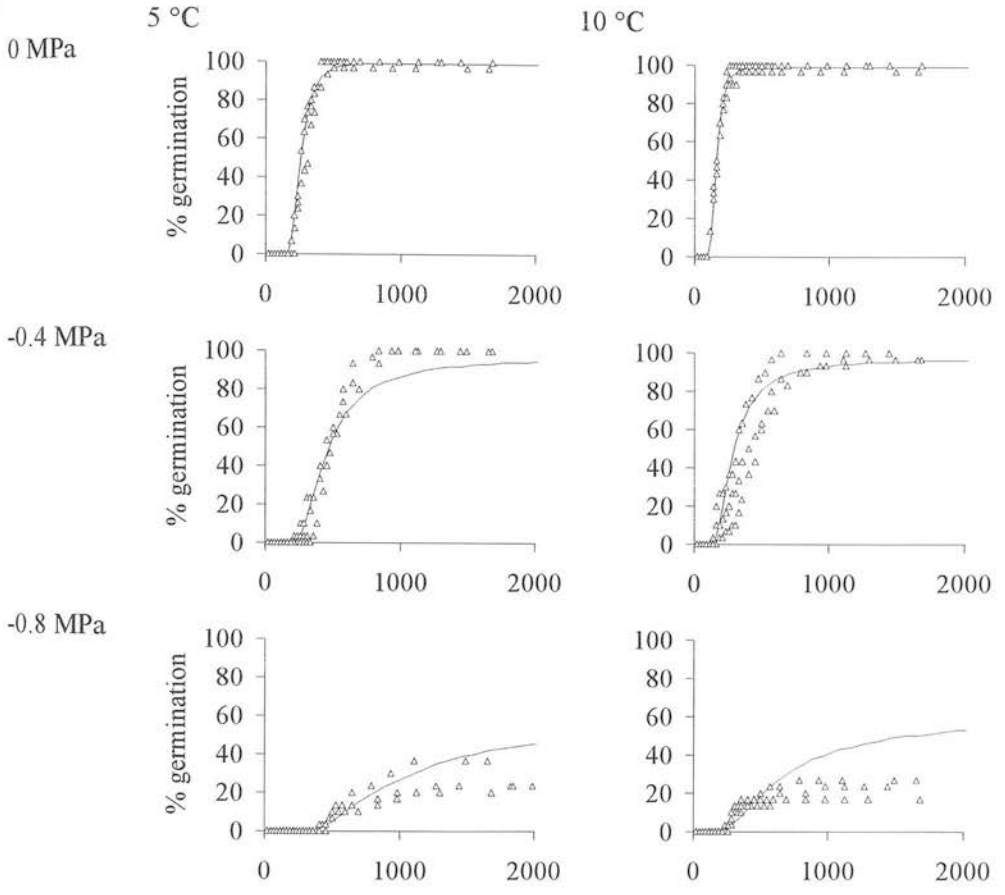


Figure 7.5: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Northumberland population of *Galium aparine* at sub-optimal temperatures following exposure to light at initiation. $r^2 = 0.77$. n = 3.

Figure 7.5 shows that for the Northumberland population, the start of germination tends to be reasonably well fitted in all combinations of temperature and water potential. However at lower water potentials the speed of germination is poorly fitted for the Northumberland population and at –0.8 MPa the hydrothermal model overestimated final percentage germination, largely through failure to asymptote.

Figure 7.6 shows that for the Northern Ireland population, the estimated start of germination was too early. It is difficult to determine how well the model estimates the speed of germination because of the ill fitted start to germination, but again at–0.8 MPa the hydrothermal model overestimated final percentage germination, largely through failure to asymptote.

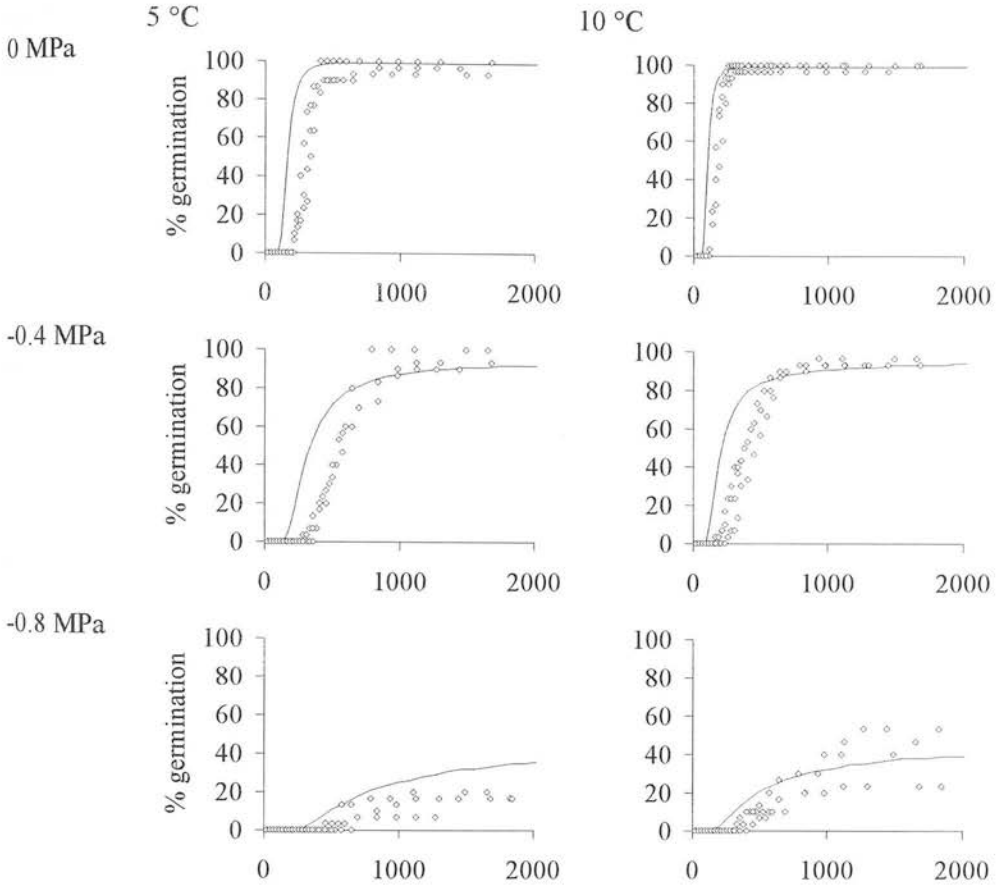


Figure 7.6: Estimated germination time courses from the hydrothermal time model compared with experimental results for the Northern Ireland population of *Galium aparine* at sub-optimal temperatures following exposure to light at initiation. $r^2 = 0.80$. n = 3.

7.5. Discussion

Three contrasting populations were used in this investigation of the effects of temperature and water potential on the germination of *G. aparine*. The data collected showed that temperature and water potential interacted to effect both the timing and duration of *G. aparine* germination and final percentage germination. There were also significant differences between populations largely in final percentage germination, but populations responded differently to water potential. This again illustrates the need to consider population differences and treat generalisations about species behaviour with caution.

In contrast to previous experiments, the seeds used were produced in a common environment with the aim of minimising the environmental component of variation, allowing differences between populations to be largely ascribed to genetic differences. The use of even-aged

seeds produced in a common environment contrasts with previous work considering the effect of temperature and water potential on the germination of weed seeds (Grundy, 1997).

Given that the three even-aged seed populations were produced in a common environment, it is apparent that the populations differed in dormancy and that this difference is likely to have a largely genetic component. The Northumberland and Northern Ireland populations exhibited minimal dormancy (germinated to a high percentage across the range of constant temperatures) whilst the North Yorkshire population exhibited higher levels of dormancy. It is speculated that this higher level of dormancy may have evolved under selection pressures associated with earlier cultivation and more intensive crop production in the North Yorkshire. However, despite differences in dormancy, there were general effects of temperature and water potential on the relative number of seeds germinating and germination rate.

7.5.1. Seed germination and temperature

Final germination percentage tended to be maximised between 5 and 10 °C and decreased at 15 and 20 °C. This is similar to observations by Froud-Williams (1985) who reported that optimal germination of field populations of *G. aparine* was between 9 and 12 °C, although this same paper quoted an upper temperature limit of 15 °C. Sjostedt (1959, in Malik & van den Born, 1988) reported that temperatures greater than 20 °C reduced the final percentage germination of freshly harvested seeds, with optimal germination at temperatures between 12 and 15 °C. Kurth (1967 in Malik & van den Born, 1988) reported a wider optimal temperature range from 0.5 to 12 °C. Grime *et al.* (1981) stated that *G. aparine* germination occurs between 6 and 26 °C, whereas Lauer (1953 in Malik & van den Born, 1988) stated a range of 2 to 20 °C. It is clear that there is substantial variation in both the range of temperatures within which *G. aparine* germination occurs and the range of temperatures that maximise germination. This may be part attributed to differences between populations, but it is clear that the optimal temperature range for *G. aparine* is lower than that recorded for *Stellaria media* (Grundy, 1997 and Chapter 6) and likely includes temperatures less than 5 °C.

Temperature also had an effect on the speed of germination, which increased as temperature increased from 5 to 15 °C. This was to be expected as the reactions and processes required for germination are likely to happen at a faster rate at higher temperatures and a similar

effect of temperature on speed of germination was recorded for *S. media*. There were generally limited differences in germination rate between 15 and 20 °C.

7.5.2. Seed germination and water potential

Germination percentage tended to decrease as water potential decreased. However it was clear that germination percentage at –0.4 MPa was only reduced at supra-optimal temperatures (15 °C or higher). This supports the observations of van der Weide (1993) who recorded reduced final percentage germination (emergence) at low soil moisture contents at 14 °C, but not 8 °C. It is suggested that this greater robustness of germination response to small reductions in water potential compared to *S. media* (Grundy, 1997 and Chapter 6) may be related to the greater seed size of *G. aparine*. Supporting this viewpoint, is interesting to note that at –0.8 MPa and at the higher sub-optimal temperatures, germination of the smallest seeded Northumberland population appeared to be particularly reduced. Malik & van den Born (1988) recorded the effect of water potential (as given by graded PEG solutions) on final percentage germination for the related, but smaller seeded species *Galium spurium* and showed that final germination percentage was reduced at –0.25 MPa and no germination occurred at water potentials less than –0.75 MPa. Given that *G. aparine* germination was recorded at -0.8 MPa (except for the Northumberland population at 20 °C), it would therefore appear that *G. aparine* may be more tolerant than *G. spurium* of low water potentials.

Low water potentials also resulted in reduced germination rate and this was consistent with the results recorded for *S. media* (Grundy, 1997 and Chapter 6) and for many crop plants (Gummerson, 1986; Dahal & Bradford, 1990).

7.5.3. Seed germination and the hydrothermal model

The hydrothermal time model proposed by Gummerson (1986) suggested that seed germination responses according to both temperature and water potential could be combined into a single expression on a hydrothermal time basis. As described in Chapter 6, this approach has been applied, with some success, to crop plants (Gummerson, 1986; Bradford, 1995). Grundy (1997) outlined the main problems of applying this analysis to predict weed germination time courses, namely that weed seeds tend to be more variable than crop plants. In predicting field germination, this variation in weed seed germination may be a

consequence of seed age, seed history and seed population genetic structure and it should be noted that in this analysis attempts have been made to eliminate the problem of seed age and seed history.

There have been no previous attempts to apply the hydrothermal time model to populations of *G. aparine*. The only previous application of this analysis to weed populations was Grundy (1997) working on *Stellaria media* and this was discussed in the previous chapter. Grundy (1997) and the previous chapter attributed many of the problems in applying the hydrothermal time model to *S. media* to the problem of seed dormancy, however it is clear that in this case, despite near 100 % germination for two of the three populations, model application remained problematic.

The first problem related to the definition of sub-optimal temperatures to include only data collected at 5 and 10 °C, which limited application of the model to these lower temperatures and excluded half the information collected in the experiment. Consequently, although linear regression and probit regression analysis could be applied to such data, it was clear that the results from these analyses were based only on a small number of data points. It is not clear whether limited data was the reason for the probit method failing to fit a base temperature to the North Yorkshire population. Although linear regression estimated a base temperature for this population, the temperature estimated was 10 °C lower than the lowest temperature used in the experiment and this extreme extrapolation limits confidence in these results. The base temperature estimated by linear regression for the other two populations of approximately -1.8 °C contrasted with the only published value for a base temperature for *G. aparine* of 2.5 °C (van der Weide, 1993). This was derived from application of a thermal time model to summarise the germination patterns of a single population of *G. aparine* and the total thermal time requirement was given as 75.2 degree days. Although there was no better agreement with base temperatures estimated by the probit method, because this method estimated a single value, it was deemed preferable and used to develop the hydrothermal time model, albeit restricted to two of the three *G. aparine* populations.

The base temperature estimated by the probit method for the Northumberland population was lower than that for the Northern Ireland population and it is suggested that this might reflect climatic differences with milder winters in the west and colder winters in the east of the UK. These base temperatures tended to be lower than those calculated for *S. media* (Chapter 6) suggesting that *G. aparine* is physiologically active at lower temperatures.

In Table 7.6 the values for the hydrothermal time constant varied from 1105 to 1985 MPa °C h^{-1}. This tended to be higher than those estimated for *S. media* in Chapter 6, but less than those predicted by Grundy (1997), also for *S. media*. Differences between populations were consistent with the Northumberland population, the populations that tended to germinate quicker in any given set of conditions, having the lowest hydrothermal time constant.

Ignoring restrictions to sub-optimal temperatures and failure to fit data from one of the three populations, the usefulness of the hydrothermal time model is demonstrated in Figure 7.5 and Figure 7.6. The onset of germination tended to be well fitted for the Northumberland population (all conditions), but not for the Northern Ireland population. It is not clear why the hydrothermal time model fitted the onset of germination too early, although it is possible that this might reflect the more protracted nature of germination for this population. In terms of the hydrothermal time model this would tend to suggest that the base temperature could be too low and/or the hydrothermal time constant too low. Overall, the hydrothermal time model tended to fit the speed of germination, especially in water. However at lower water potentials, the model tended to underestimate the speed of germination and overestimate final percentage germination.

The results for *G. aparine* further demonstrated the two main problems with the hydrothermal time model. These are that no statistical methods have previously been used to calculate confidence intervals for either model output or parameter estimates and that failure to asymptote results in poor model fits, especially at low water potentials where final percentage germination is reduced. Again it is clear that a parameter to estimate final percentage germination needs to be included in the model. It should also be noted that additional criticisms were levelled in Chapter 6 related to the lack of general consistency in the model and difficulty in identifying how the model could be extended to account for further environmental factors. These criticisms support the decision to adopt an alternative modelling framework and this is described in Chapter 11.

7.6. Conclusions

Final percentage germination and the timing of germination differed significantly between populations of *G. aparine* according to temperature and water potential. There were also a

large number of significant interactions between environmental factors. In general, final percentage germination was maximised at 5 and 10 °C and decreased at higher temperatures. Germination was also more protracted at 20 °C. Water potentials of -0.4 MPa reduced final percentage germination only at higher temperatures (> 15 °C), but water potentials of -0.8 MPa reduced final percentage germination at all temperatures. The time to the onset of germination also increased with lower water potentials. There was an indication that final percentage germination of the smaller seeded population was disproportionally reduced at -0.8 MPa and higher temperatures.

Application of the hydrothermal time model was restricted to germination at temperatures less than 10 °C. Failure to fit a base temperature to one of the three populations (by probit regression analysis) also limited the utility of the model. Despite near complete germination in optimal conditions for the two *G. aparine* populations to which the model was fitted, the model consistently overestimated germination at low water potentials and for one population also fitted the start of germination too early. This failure to asymptote correctly together with the lack of confidence intervals, questions the utility of this model for predicting weed population germination time courses.

Chapter 8. The effect of fluroxypyr on seed production and seed germination for contrasting populations of *Stellaria media*.

8.1. Summary

An experiment was conducted to investigate the effect of reduced rate herbicide applications on subsequent seed production and seed germination for three contrasting populations of *Stellaria media*. Reduced rate herbicide applications were selected as an easy method of manipulating plant growth for investigations of variation in seed characteristics, according to differences in the maternal environment. They also represent an integrated approach to weed control in arable cropping. The herbicide used was fluroxypyr and it was applied at the 8-10 leaf growth stage.

Applications of fluroxypyr markedly affected plant growth and significantly reduced seed production for two of the three populations. For these same two populations, the seeds produced following herbicide application were significantly reduced in size and low levels of germination were recorded in tests conducted at a range of temperatures. It was suggested that this reduced germination was associated with smaller seed size and not with persistent effects of herbicide chemistry. Effects of herbicide application on total seed production, seed size and seed germination were not significant for the Perthshire population and this was associated with less marked effects on plant growth and characteristically late flowering and low reproductive effort.

Herbicide application prior to seed production only effected subsequent germination extent, not germination synchronicity or the speed of germination. Significant differences were recorded between populations and these were consistent with other reports in this study (Chapters 2, 6 & 10).

Populations varied in susceptibility to the herbicide fluroxypyr and the different responses of populations to herbicide application in terms of plant growth and subsequent seed production, seed size and seed germination demonstrated the importance of considering intraspecific variation in studies of weed ecology. It also demonstrated the pronounced

effect that differences in the maternal environment can have on subsequent seed characteristics.

8.2. Introduction

Herbicides are developed to control weeds by either complete eradication of the growing weed plants or by reduction in biomass. The primary aim is to increase crop yields by reducing the intensity of competition between the crop and weed plants (Attwood, 1985). In some cases, herbicides are also used to reduce weed interference with machinery at harvest and to limit weed seed contamination of the harvested crop (Attwood, 1985). However, it is clear that the main interest in the commercial development of herbicides is in the immediate effect that compounds have on weed biomass and little consideration is paid to secondary effects of herbicide application for subsequent generations of weed plants. These effects are likely to become more important as low dose herbicide applications are increasingly promoted to achieve economic savings and environmental benefits (e.g. Fisher, Davies & Whytock, 1993; Ogilvy *et al.*, 1995; Tait & Pitkin, 1995). This is because the use of low dose herbicides may increase the likelihood of weed survival to set seed (Fisher *et al.*, 1993; Cook, Hill & Green, 1996; Cook & Clarke, 1997). It is therefore important to understand the possible secondary effects of herbicide application on future weed populations.

The secondary effects can be divided into four different types of effect. The most obvious effect is that of herbicide application on weed seed production and the number of seeds returned to the soil for the next weed generation. Additional effects may be shown in physical seed characteristics, seed germination and in subsequent patterns of plant growth.

The effect of herbicide application on weed seed production has been demonstrated in a number of studies (e.g. Fawcett & Slife, 1978a; Rasmussen, 1993; Hald, 1993; Andersson, 1994a, b, 1995). Seed production is a function of plant biomass, reproductive effort (that is the proportion of biomass allocated to seed production), seed size, plant longevity and plant phenology (Harper, 1977). Herbicide application eradicates or reduces weed biomass and this usually limits weed seed production. The extent to which seed production is actually reduced interacts with the other factors listed above. For example, Hald (1993) showed that seed production in *Thlaspi arvense* was a linear function of plant dry weight, and that this was independent of the herbicide (isoproturon) rate applied. This linear relationship between seed production and plant dry weight was also described for *Chenopodium album* by

Rasmussen (1993) and for *Viola arvensis* by Grundy, Froud-Williams & Boatman (1995). However, Rasmussen (1993) also demonstrated year to year variability in seed production per unit area and herbicide response. It is clear that reduced seed production limits the number of seeds returned to the soil seedbank and this has important implications for the size of future weed populations.

Seed production interacts with seed size, with a trade-off between seed number and seed size. Herbicide application has been shown to effect seed size in some instances. Champion, Froud-Williams & Holland (1997, 1998) recorded an increased proportion of smaller seeds in field populations of *Veronica persica* treated with fluroxypyr and Andersson (1996) recorded significantly reduced seed size for *Galium spurium, Fallopia convolvulus* and *T. arvense* following applications of full dose tribenuron-methyl, though results were specific according to weed size at treatment. In contrast Hume & Shirriff (1989) reported that *Chenopodium album* seed size was increased by treatment of parent plants with low doses of 2, 4 D and Andersson (1994b) recorded an increased 1000 seed weight for *Myosotis arvensis* treated with MCPA. Grundy *et al.* (1995) reported no significant effect on seed weight for *Viola arvensis* treated with a mixture of clopyralid, fluroxypyr and ioxynil. Changes in seed size may be of ecological significance through effects on patterns of seed dispersal, with smaller, lighter seed potentially dispersing over greater distances and larger, heavier seed subject to lower probabilities of soil incorporation and burial (Thompson, Band & Hodgson, 1993). Changes in seed size may also affect the likelihood of seed predation (Reader, 1993) and may be associated with changes in seed viability and seed germination characteristics (Stanton, 1984).

Changes to seed germination characteristics for seed produced following herbicide application are likely to be a function of the specific herbicide applied and the rate and timing of application. For example, Shuma *et al.* (1995) studied the effect of glyphosate application at four different rates and at four stages of seed development in *Avena fatua*. They showed that application at anthesis completely prevented the formation of viable seeds and applications at all rates, ten days after anthesis reduced seed viability. It was also shown that no viable seeds were produced when rates greater than 1.76 kg active ingredient (a.i.) ha^{-1} were applied at all growth stages. However, it should be noted that seed viability was not assessed directly, only by comparison with treatments designed to maximise germination. The mechanism by which glyphosate reduced seed viability was not identified. Andersson (1996) showed that the germination and seed viability (assessed by

tetrazolium tests) of *G. spurium* and the germination of *T. arvense* were reduced by low dose applications of tribenuron-methyl and additionally, the germination of *F. convolvulus* was reduced by low dose applications of MCPA. Significant variation in response was recorded with the actual effect greatly depending on the weed growth stage at application. For *G. spurium*, but not *F. convolvulus* or *T. arvense*, reduced seed germination and to a lesser extent, seed viability, were correlated with reductions in seed size following herbicide treatment (Andersson, 1996). Champion *et al.* (1998) also showed that an increased proportion of small seeds could explain reductions in germination for seed produced by fluroxypyr treated *Veronica persica*. Both Andersson (1996) and Champion *et al.* (1998) suggested that lower levels of seed germination in smaller seeds may be related to a decrease in stored seed nutrients. It should be noted that herbicide effects on germination timing have not been described, despite the importance of germination timing in determining individual growth and development and the outcome of competition (Benjamin, 1990; Ghersa & Holt, 1995; Fenner, 1995).

There have been only a limited number of investigations into changes in plant growth characteristics for plants emerging from seeds produced following herbicide application. It is likely that changes in plant growth characteristics are also complex functions of the specific herbicide applied and the rate and timing of herbicide application. Grundy *et al.* (1995) harvested *Viola arvensis* seeds from a field trial following application of a mixture of clopyralid, fluroxypyr and ioxynil at either full, 1/2, 1/4 or 1/8 of the manufacturers' dose rate. Subsequent experiments showed that herbicide dose in the maternal generation affected plant height in the second generation and this was also shown to interact with the nitrogen fertiliser application rate, resulting in greater reductions in offspring plant height at the higher level of nitrogen fertilisation. Similarly Andersson (1996) showed that low dose applications of tribenuron-methyl at intermediate growth stages reduced subsequent *G. spurium* seedling shoot biomass and root biomass, whilst Champion *et al.* (1997) showed that low dose applications of fluroxypyr reduced *V. persica* height. In both cases these effects were attributed to reductions seed size following herbicide application. The reduction of weed biomass in the second generation as a consequence of herbicide application pre-seed formation has implications for the level of future weed seed production and the relative competitive ability of the weed growing within the crop (Stanton, 1984).

It is clear that low dose herbicide application can have a broad range of effects on weed seed biology with important implications for the behaviour and control of future weed

populations. It was decided to investigate the effects of applying a range of low doses of herbicide on the seed production and seed germination characteristics for three contrasting populations of *Stellaria media*. This included investigations of the effects of herbicide application on subsequent patterns of germination timing. Additional information on seedling growth characteristics was collected, but these will not be presented. The selected herbicide was fluroxypyr (Starane 2: Dow Agrosciences) and it was chosen because it is a widely used broad-spectrum herbicide, with a specific recommendation for the control of *S. media*. Fluroxypyr is applied post-emergence to control a wide range of broad-leaved weeds in cereal crops and grass and UK farmers often reduce doses below that recommended by the manufacturer in order to reduce costs (Ken Davies, pers. comm.). It is an aryloxyalkanoic acid herbicide and it is applied by foliar spraying. Fluroxypyr is translocated and accumulates within the plant to produce auxin-type responses. Plant growth is disrupted by deregulation of cellular growth processes following binding of fluroxypyr to plant cell auxin receptor sites. Fluroxypyr also interferes with nitrogen metabolism and enzyme production (Environmental Protection Agency, 1998).

8.3. Methods

8.3.1. Seed used

The experiment initially used mature seed harvested in August 1996 from three populations of *Stellaria media* (as identified in Chapter 2). The seed was harvested from plants grown in an unheated glasshouse with seed set under muslin to prevent cross-pollination. It should be noted that use of seed replicated in a common environment was to minimise initial differences between populations related to differences in maternal environments. Seeds were stored dry in an incubator maintained at 10 °C (± 2 °C) prior to the experiment.

8.3.2. Herbicide treatment, plant growth and seed production

In November 1997, 150 pots (17.8 cm diameter) were filled with a standard peat and perlite mixture (defined in Appendix 1). 50 pots were allocated to each population and the pots were labelled with the population and treatment code. Ten seeds of *S. media* from the named population were surface sown into each pot and the pots were placed with populations grouped together in two blocks in an unheated greenhouse. A distance of approximately 2 m separated the populations within the blocks to minimise cross pollination between populations. Pots were watered regularly and the seedlings were thinned to a single plant per

pot on emergence. Seedling height and width across the second two leaves was recorded at four true leaves.

On 11 March 1998, plants were moved outside for herbicide treatment. The weather was dry, wind speeds were low and air temperature was average (~8 °C). The plants had approximately eight to ten true leaves and no buds or flowers were recorded. Ten plants per population were sprayed with one of five treatments: 1/2 dose (100 g a.i. ha^{-1}), 1/4 dose (50 g a.i. ha^{-1}), 1/16 dose (12.5 g a.i. ha^{-1}) or 1/64 dose (3.125 g a.i. ha^{-1}) fluroxypyr (Starane 2: Dow Agrosciences) and a control set was sprayed with an equal volume of tap water. Treatments were applied by trained staff using a Cooper Pegler CP3 knapsack sprayer with a 1.8 m boom, equipped with 6 Orange Lurmark nozzles (F80/0/80/2 Medium). This produced a medium spray quality at 2.75 bar, producing a water volume rate of 230 l ha^{-1}.

Seedling survival and growth patterns were recorded four and eight weeks after treatment. As the plants grew, canes and twine were used for support and to restrict interaction between neighbouring pots. Seeds were harvested by hand at approximately monthly intervals after initiation of flowering. Seeds were collected at maturity, just prior to capsule splitting and seed dispersal. Harvested seeds were placed in a tray lined with absorbent paper and labelled with population, treatment codes and date of harvest. The seeds were then left to dry at room temperature for c. 2 weeks. The seeds were then hand cleaned (using graded sieves) and transferred in labelled, sealed paper envelopes to a cooled incubator maintained at 10 °C, with silica gel used to maintain low relative humidity. Cleaned seeds from each harvest were weighed separately and the total seed weight of seeds harvested per plant was calculated.

On the last harvest date (when the treated plant had senesced), the remaining above ground plant material was harvested and placed in a labelled paper bag. The remaining above ground plant material was dried in an oven at 100 °C for 24 hours and then weighed.

Differences between populations and between treatments were analysed by analysis of variance (Genstat 5). Analysis of variance and correlation between above ground vegetative dry weight and total seed production were restricted to individual plants that produced seed. Zero values for surviving plants that failed to set seed were not included.

Seeds harvested within four weeks from mid July and mid August were bulked together by population and treatment and used in subsequent germination and seedling growth experiments. The only exception was for seed produced following 1/2 fluroxypyr application to the Leicestershire population for which seed produced in early September was used. This was because seed production was delayed in this population following this herbicide application and insufficient seed was available earlier.

8.3.3. Seed size and weight

Seed size and weight characteristics were determined from samples of the same seed used in the germination experiments (seed harvested between mid-July and mid-August 1998). Seed diameter was assessed by using callipers to measure individual seed diameter to the nearest 0.05 mm. There were 10 replicates for each population x herbicide treatment. Seed weight was assessed by weighing five seed samples, with three replicates for each population x herbicide treatment. Analysis of variance (Genstat 5) was used to identify differences in seed diameters and five seed weight between populations and treatments.

8.3.4. Seed germination

Starting in January 1999, the germination characteristics of the seed harvested from the control and herbicide treated plants between mid July and mid August 1998 were assessed at four constant temperatures (5, 10, 20 and 30 °C) following an initial exposure to red light and subsequent intermittent exposure to very low levels of green light.

For each germination test, 50 seeds were placed on a double layer of filter paper (Whatman no. 181) in a 9 cm plastic Petri-dish. There were three replicates for each population x herbicide x temperature treatment, established at approximately weekly intervals to counter the lack of replication in incubators. Under a green safe light, each Petri dish received 10 ml of distilled water. The green safe light was given by an Osram L 36W/30 fluorescent tube and diffuser covered with two layers of dark yellow-green Cinemoid filter (Lee filters #90), giving a photosynthetically active photon flux density (PPFD) of 0.51 ± 0.19 $\mu mol\ m^{-2} s^{-1}$. Petri-dishes were then sealed in a self-seal clear polythene bags to minimise water loss by evaporation. Petri-dishes were then exposed to red light for 15 minutes. The red light was given by an Osram L 36W/30 fluorescent tube and diffuser covered with two layers of bright red Cinemoid filter (Lee filters #26), giving a PPFD of 3.84 ± 0.71 $\mu mol\ m^{-2} s^{-1}$ (PPFD

recorded by quantum sensor and a Campbell CR10 datalogger). Then, again under the green safe light, these Petri dishes were randomly placed in sealed cardboard boxes wrapped in black polythene and lined with bubble wrap, with a separate box for each temperature. The boxes were then removed to unlit incubators (Gallenkamp/Cryotechnics cooled) maintained at the defined constant temperatures of 5, 10, 20 and 30 °C (± 2 °C). Germination was assessed under the green safe light, after 24, 36 and 48 hours, then daily for the next eight days and then at less frequent intervals until germination ceased. Seed germination was defined as radicle emergence to greater than 1 mm and germinated seeds were removed from Petri dishes when recorded.

For statistical analysis, a Gompertz curve was fitted to cumulated seed germination data by least squares for each Petri dish (Genstat 5). The Gompertz curve was poorly fitted to data sets where seed germination was recorded after the initial 24 hours and an additional time point with zero germination recorded at 10 hours was inserted to improve curve fitting. The Gompertz curve was defined as detailed in Chapter 2.

The Gompertz curve was also used to estimate time to 50 % germination (t_{50}) for each data set. Analysis of variance (Genstat 5) was used to identify differences in the fitted parameters of the Gompertz curve and estimated t_{50} between populations, treatments and temperatures. Data sets that were poorly fitted by the Gompertz curve ($r^2 < 0.9$) were excluded from the analysis.

8.4. Results

8.4.1. Plant growth and seed set following fluroxypyr treatment

Following spraying on 11 March 1998, observations 28 days later showed that 1/2 dose fluroxypyr reduced the number of plants remaining from 10 to four plants for the Perthshire population and from 10 to seven for the Caithness population. Quarter dose fluroxypyr reduced the number of plants remaining from 10 to nine plants for the Caithness population only. There was no reduction in the number of seedlings remaining following other treatments and all treated plants from the Leicestershire population survived. Application of 1/4 and 1/2 dose fluroxypyr resulted in marked die back of treated plants with characteristic leaf curl and root proliferation. Some leaf curl and root proliferation were also observed in plants treated with 1/16 dose fluroxypyr. Figure 8.1 shows that 8 weeks after fluroxypyr treatment, plants from the Caithness and Leicestershire populations were observed

flowering. Flowering was more frequent in the Caithness population, but for both populations, no flowering was recorded 8 weeks after treatment for plants previously treated with 1/2 dose and the frequency of flowering following application of 1/4 dose fluroxypyr was reduced. Similarly, Figure 8.2 shows that extensive rooting (as measured by roots extending outwith pots) was restricted to control plants and plants treated with very low doses of fluroxypyr.

Seed was harvested at regular intervals prior to plant senescence. The record of seed collections shows that seed production was highest in July for the Leicestershire and Caithness populations and in August for the Perthshire population.

Figure 8.3 shows significant differences between populations and between treatments and interactions between these factors for the total weight of seed produced by individual plants. The total weight of seed produced by the Caithness population was greater than that produced by Leicestershire population, which in turn was greater than that of the Perthshire population. There were no significant differences between treatments in the total weight of seed produced for plants from the Perthshire population, although the mean total weight of seed produced by plants treated with 1/2 dose fluroxypyr was less than half that of the control. For the Leicestershire and Caithness populations the total weight of seed produced per plant was significantly reduced for plants treated with 1/2 dose fluroxypyr compared with the control. For the Caithness population there were no significant differences between the other treatments. For the Leicestershire population the total weight of seed produced reduced with increasing dose of fluroxypyr.

Figure 8.4 shows significant differences between populations and between treatments for the dry weight of above ground vegetative material following final harvest. The dry weight of above ground vegetative material after final seed harvest for the Perthshire population was greater than that for the Leicestershire population, which in turn was greater than that for the Caithness population. For all three populations there were no significant differences between control, 1/64 and 1/16 doses of fluroxypyr in above ground vegetative dry weight. Above ground vegetative dry weight was significantly reduced in all three populations following application of 1/2 dose fluroxypyr. Following application of 1/4 dose fluroxypyr, above ground vegetative dry weight was significantly reduced in the Leicestershire and Perthshire populations, but not in the Caithness population.

Figure 8.5 shows the relationship between above ground vegetative dry weight and total seed weight. It is interesting to note that there was better linear relationship between above ground dry weight and total seed weight for the Perthshire population, but that this was not evident for the Leicestershire and Caithness populations.

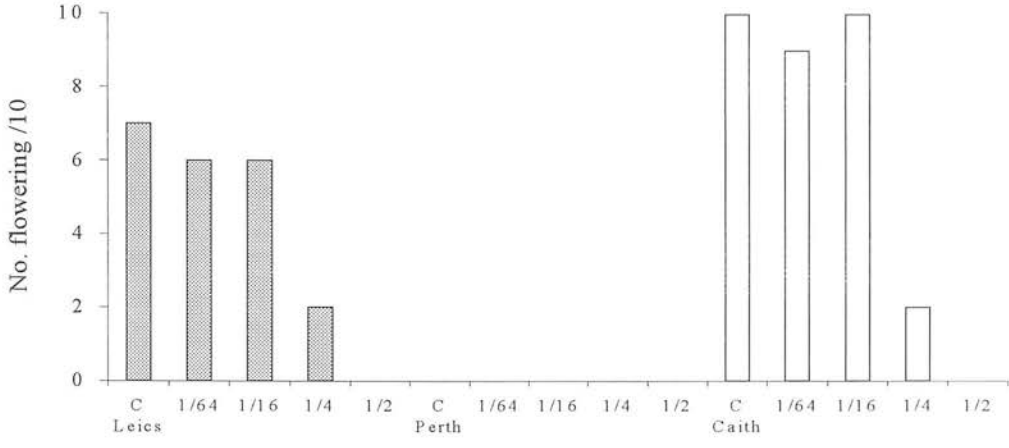


Figure 8.1: Number of individuals of *Stellaria media* /10 flowering, 8 weeks after application of fluroxypyr at a range of doses.
C = Control: Herbicide doses as fraction of full rate.

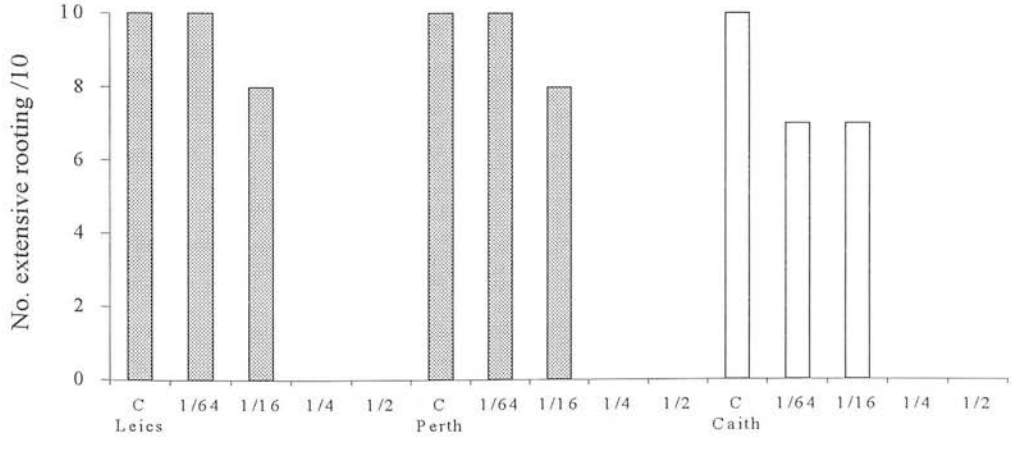
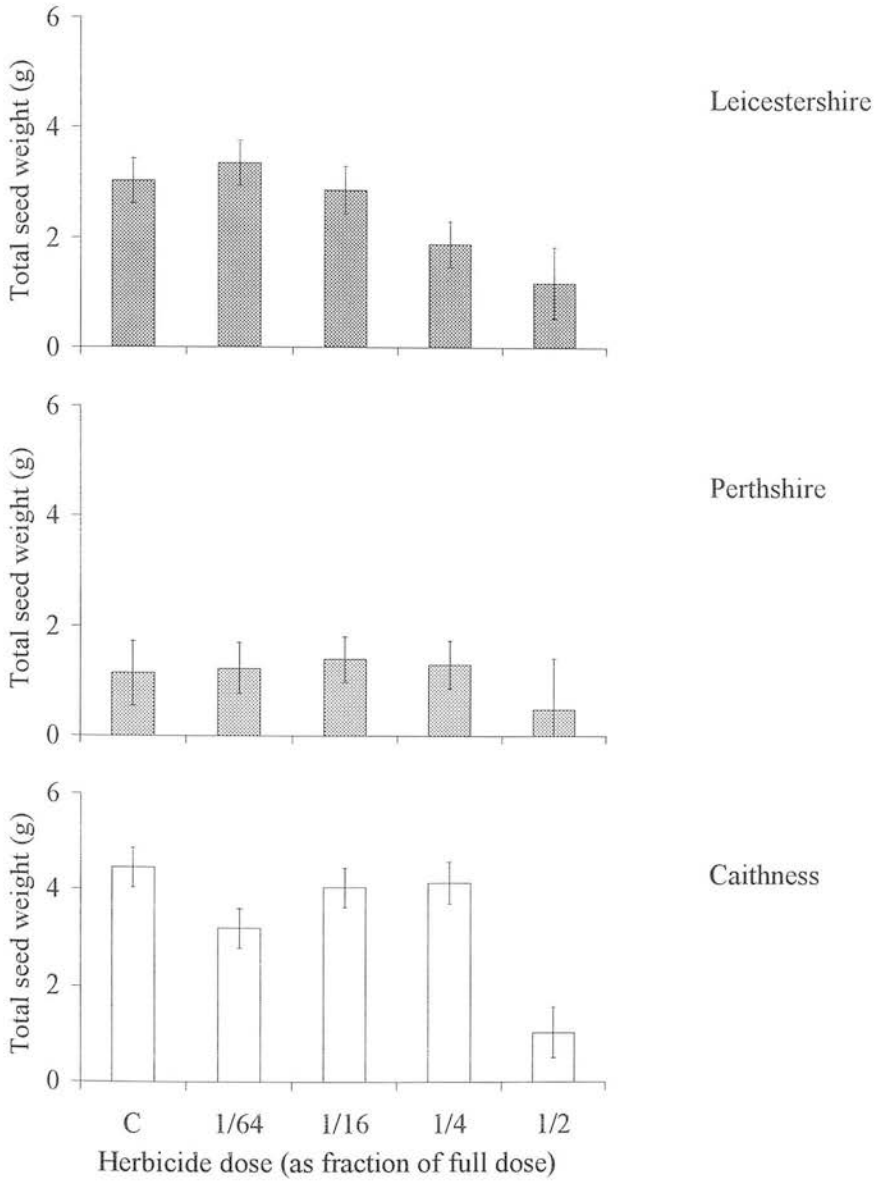


Figure 8.2: Number of individuals of *Stellaria media* /10 with extensive rooting (as measured by roots extending outwith pots, 8 weeks after application of fluroxypyr at a range of doses.
C = Control: Herbicide doses as fraction of full rate.

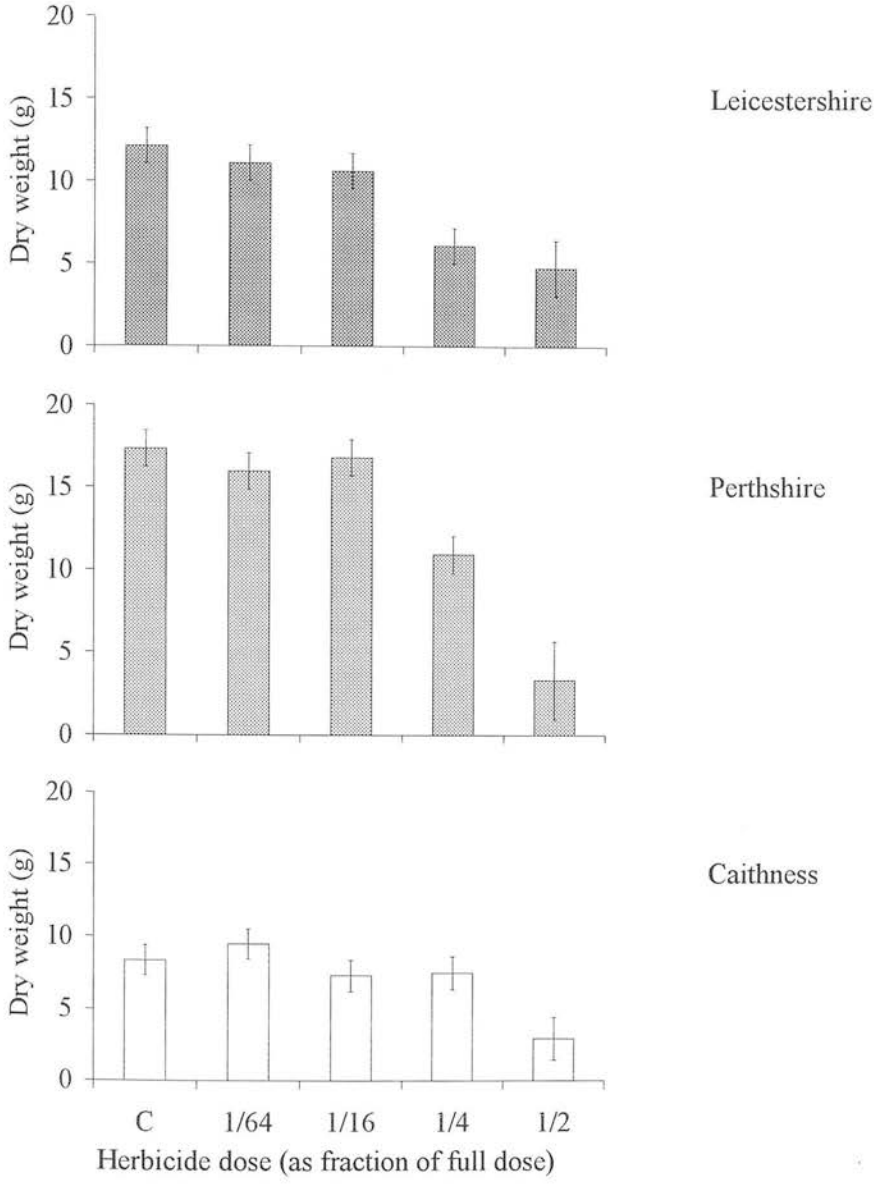


F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	38.05	***
Herbicide dose	4	11.33	***
Population x herbicide dose	8	2.70	**
Residual	107		

(28 missing values)

Figure 8.3: Total weight of seed produced by individual plants from three populations of *Stellaria media* treated with fluroxypyr at reduced doses.
Mean total seed weight per plant ± S.E.: C = Control: n = 10.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	39.08	***
Herbicide dose	4	37.79	***
Population x herbicide dose	8	4.13	***
Residual	111		

(24 missing values)

Figure 8.4: Total dry weight of above ground vegetative material after final seed harvest for three populations of *Stellaria media* treated with fluroxypyr at low doses. Mean above ground vegetative dry weight per plant ± S.E.: C = Control: n = 10.

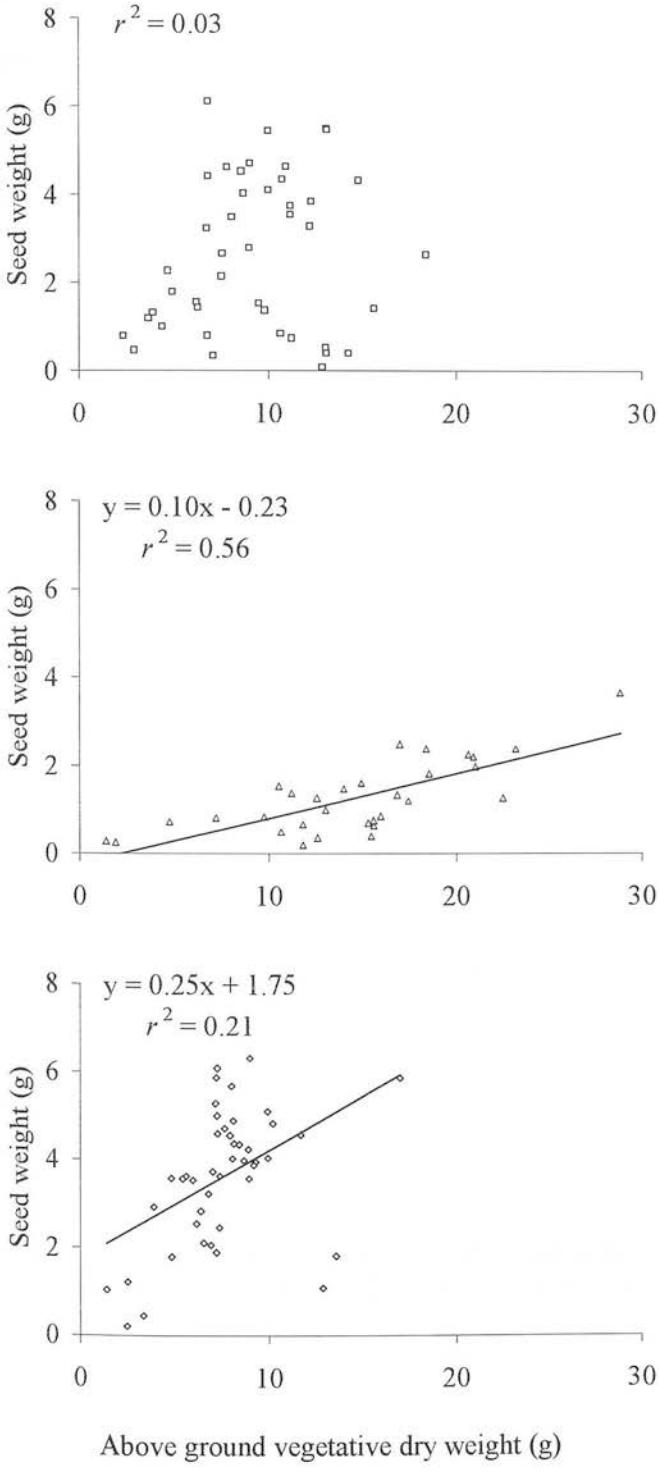


Figure 8.5: Relationship between above ground vegetative dry weight and total seed weight for *Stellaria media* populations.

8.4.2. Seed size and weight following fluroxypyr treatment

Table 8.1 shows mean seed diameters for each population according to herbicide treatment. Seed diameters varied significantly between populations and between treatments, although the effect of previous herbicide treatment varied according to population. Seed diameter was significantly wider for the Caithness population. For the Caithness and Leicestershire populations, seed diameters for seed produced in the control treatment (C) were significantly wider than those for seed produced following application of 1/2 dose fluroxypyr.

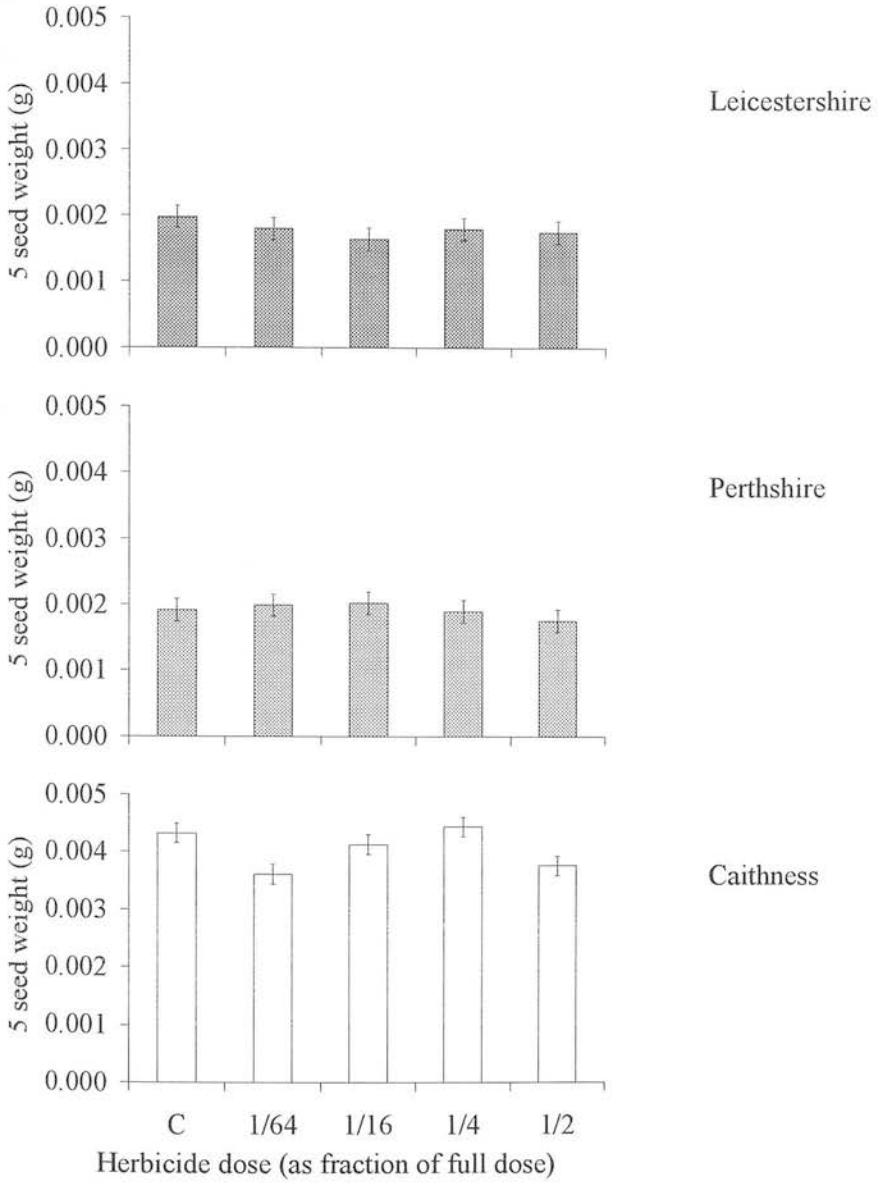
Figure 8.6 shows mean five seed weights for each population according to herbicide treatment. Five seed weights varied significantly between populations, but no significant differences were recorded for previous herbicide treatment. Five seed weight was highest for the Caithness and lowest for the Leicestershire population. Individual mean seed weight was 0.81 mg for the Caithness population, 0.38 mg for the Perthshire population and 0.36 mg for the Leicestershire population.

Population	C	1/64	1/16	1/4	1/2	Mean
Leicestershire	0.96	0.87	0.78	0.82	0.83	0.85
Perthshire	0.82	0.85	0.81	0.84	0.80	0.82
Caithness	1.26	1.14	1.17	1.24	1.10	1.18
Mean	1.01	0.95	0.92	0.96	0.91	0.95

F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	151.67	***
Herbicide dose	4	5.06	***
Population x herbicide dose	8	2.43	*
Residual	135		

Table 8.1: Mean seed diameters for seeds produced following application of fluroxypyr at low doses to three populations of *Stellaria media*.
C = Control. n = 10.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	286.63	***
Herbicide dose	4	2.15	-
Population x herbicide dose	8	1.63	-
Residual	30		

Figure 8.6: Five seed weights for seeds produced following application of fluroxypyr at low doses to three populations of *Stellaria media*.
Mean five seed weight ± S.E.: C = Control. n = 3.

8.4.3. Germination of seed produced following fluroxypyr treatment

Table 8.2, Table 8.3 and Table 8.4 show the mean fitted Gompertz parameters and the derived values for time to 50 % germination for each of the populations according to herbicide treatment and temperature. Table 8.5 summarises the analysis of variance for differences between populations, treatments and temperatures according to the different Gompertz parameters.

Table 8.2, Table 8.3, Table 8.4 and Table 8.5 show that germination of the Caithness population tended to be less synchronous (lower values of Gompertz parameter β) than the Leicestershire and Perthshire populations and overall germination was most synchronous at 10 °C. This was particularly pronounced for the Perthshire population. The synchronicity of germination was not affected by the herbicide treatment. Similarly herbicide treatment did not affect the speed of germination as measured by the Gompertz parameter μ and the derived value for time to 50 % germination (t_{50}). There were significant differences in the speed of germination according to temperature, with germination fastest at 20 °C and slowest at 5 °C.

There were significant differences in final percentage germination, as measured by the sum of Gompertz parameters ($\alpha + \gamma$) according to population, herbicide treatment and temperature, with significant interactions. These differences are shown in Figure 8.7. Final percentage germination was significantly higher for the Perthshire population with > 95 % germination recorded at all temperatures, except 30 °C. There were no evident herbicide treatment effects for the Perthshire population. For the Leicestershire and Caithness population final percentage germination tended to be higher for seeds produced without herbicide treatment or following application of the lowest dose (1/64) of fluroxypyr. This effect was consistent for the Leicestershire population except for seeds produced following application of 1/2 dose fluroxypyr and germinated at constant 5 or 10 °C, or for seeds produced following application of 1/4 dose fluroxypyr and germinated at a constant 30 °C. For the Caithness populations these results were consistent except for germination at 30 °C, where the significantly higher final percentage germination was restricted to seeds produced without herbicide application. It is interesting to note that final percentage germination for the Caithness population was maximised for all treatments at 30 °C. It was also noted that there was no simple relationship between final percentage germination and dose for seed produced following application of 1/16, 1/4 and 1/2 dose fluroxypyr. For the Leicestershire

population, final percentage germination tended to be lowest for seed produced following application of 1/16 dose fluroxypyr whilst for the Caithness population final percentage germination tended to be lowest for seed produced following application of 1/4 dose fluroxypyr. Figure 8.8 contrasts patterns of germination for the seeds produced without herbicide treatment and for seed produced following application of 1/4 dose fluroxypyr.

Treatment	Temperature °C	β hr^{-1}	μ hr	γ	α	t_{50} hr
C	5	0.0781	123.31	71.35	0.08	127.99
	10	0.0779	87.11	78.49	-0.18	91.86
	20	0.0907	34.02	65.33	-2.76	38.74
	30	0.0618	45.03	19.42	-0.07	51.04
1/64	5	0.0673	122.17	72.76	-0.23	127.68
	10	0.1064	87.51	84.20	0.09	90.94
	20	0.0918	35.49	58.68	-1.43	39.62
	30	0.0910	63.00	21.63	-0.80	67.62
1/16	5	0.0897	121.56	45.69	-0.05	125.66
	10	0.0777	86.54	44.81	-0.08	91.30
	20	0.0894	32.64	30.58	-1.36	37.47
	30	0.0707	37.54	12.85	-0.52	43.56
1/4	5	0.0784	117.94	57.22	-0.10	122.64
	10	0.1111	84.83	66.73	-0.10	88.15
	20	0.0899	33.00	38.04	-2.79	37.27
	30	0.0780	42.40	20.54	-0.35	47.42
1/2	5	0.0490	118.97	75.50	0.00	126.45
	10	0.0849	86.40	79.35	-0.14	90.75
	20	0.0894	32.44	47.53	-1.97	37.22
	30	0.0924	50.17	11.04	-0.57	54.96

Table 8.2: Mean Gompertz parameters for *Stellaria media* seeds from the Leicestershire population produced following fluroxypyr application and germinating at a range of temperatures following a short exposure to red light.

Treatment	Temperature °C	β hr^{-1}	μ hr	γ	α	t_{50} hr
C	5	0.0851	125.29	98.98	0.10	129.58
	10	0.1364	86.66	99.68	-0.21	89.37
	20	0.0894	32.46	103.00	-5.29	37.40
	30	0.0797	43.22	83.44	-2.01	48.25
1/64	5	0.0765	125.47	96.34	0.11	130.24
	10	0.1116	87.85	96.29	-0.06	91.15
	20	0.0888	31.69	104.41	-6.44	36.83
	30	0.0927	44.57	87.80	-0.68	48.64
1/16	5	0.0798	120.05	100.18	-0.09	124.66
	10	0.1460	82.73	98.99	-0.15	85.25
	20	0.0887	31.69	105.72	-4.97	36.59
	30	0.0899	38.68	91.68	-1.01	42.94
1/4	5	0.0892	120.49	100.54	0.20	124.57
	10	0.1338	80.98	99.94	-0.02	83.72
	20	0.0890	31.91	104.58	-5.46	36.88
	30	0.0869	44.53	81.52	-0.71	48.89
1/2	5	0.0844	114.32	98.03	0.63	118.55
	10	0.1152	74.68	97.23	0.78	77.76
	20	0.0876	30.18	107.63	-5.54	35.22
	30	0.0738	46.20	92.18	-0.16	51.20

Table 8.3: Mean Gompertz parameters for *Stellaria media* seeds from the Perthshire population produced following fluroxypyr application and germinating at a range of temperatures following a short exposure to red light.

Treatment	Temperature °C	β hr^{-1}	μ hr	γ	α	t_{50} hr
C	5	0.0509	113.95	61.03	0.03	121.14
	10	0.0736	82.76	72.53	0.09	87.72
	20	0.0917	35.06	41.85	-0.35	39.19
	30	0.0652	39.65	96.64	-1.18	45.54
1/64	5	0.0580	119.40	61.66	-0.08	125.75
	10	0.0871	80.20	70.29	0.19	84.36
	20	0.0860	35.32	53.64	-0.87	39.85
	30	0.0555	38.12	76.93	-2.00	45.41
1/16	5	0.0656	117.55	38.30	-0.03	123.15
	10	0.0832	80.02	50.02	0.71	84.19
	20	0.0917	35.12	30.90	-0.87	39.56
	30	0.0508	47.41	83.59	-0.66	54.85
1/4	5	0.0500	108.41	27.04	0.08	115.66
	10	0.1065	79.56	31.18	-0.01	83.01
	20	0.0883	38.61	20.50	0.13	42.65
	30	0.0385	51.53	66.29	-1.19	61.74
1/2	5	0.0705	123.20	42.49	-0.08	128.44
	10	0.1148	86.50	50.01	0.37	89.60
	20	0.0667	37.09	17.33	-0.70	43.46
	30	0.0613	52.29	77.91	0.10	58.24

Table 8.4: Mean Gompertz parameters for *Stellaria media* seeds from the Caithness population produced following fluroxypyr application and germinating at a range of temperatures following a short exposure to red light.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	2	24.42	***	2.09	-	670.26	***
Temperature	3	31.78	***	1176.83	***	41.05	***
Treatment	4	0.44	-	1.23	-	27.74	***
Population x temperature	6	4.42	***	1.41	-	109.14	***
Population x treatment	8	0.91	-	1.87	-	15.15	***
Temperature x treatment	12	0.91	-	0.78	-	134.71	*
Pop. x temperature x treatment	24	1.44	-	1.07	-	121.27	**
Residual	116						

(2 missing values)

Table 8.5: Summary of the analysis of variance for the differences between *Stellaria media* populations for fitted Gompertz parameters according to test temperature and herbicide treatment.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$

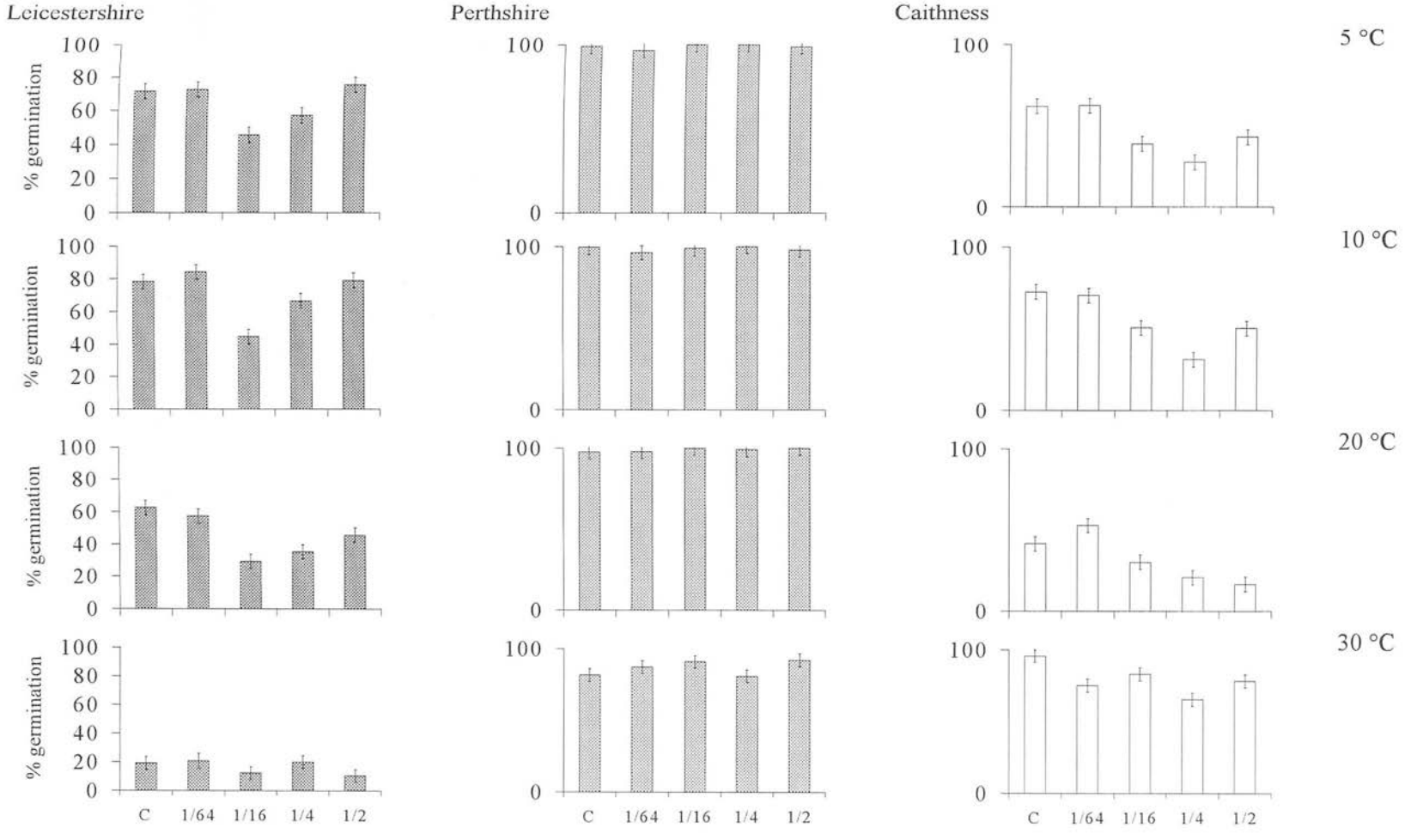


Figure 8.7: Final % germination estimated from Gompertz parameters ($\alpha + \gamma$) for *Stellaria media* seeds produced after fluroxypyr application ± S.E.

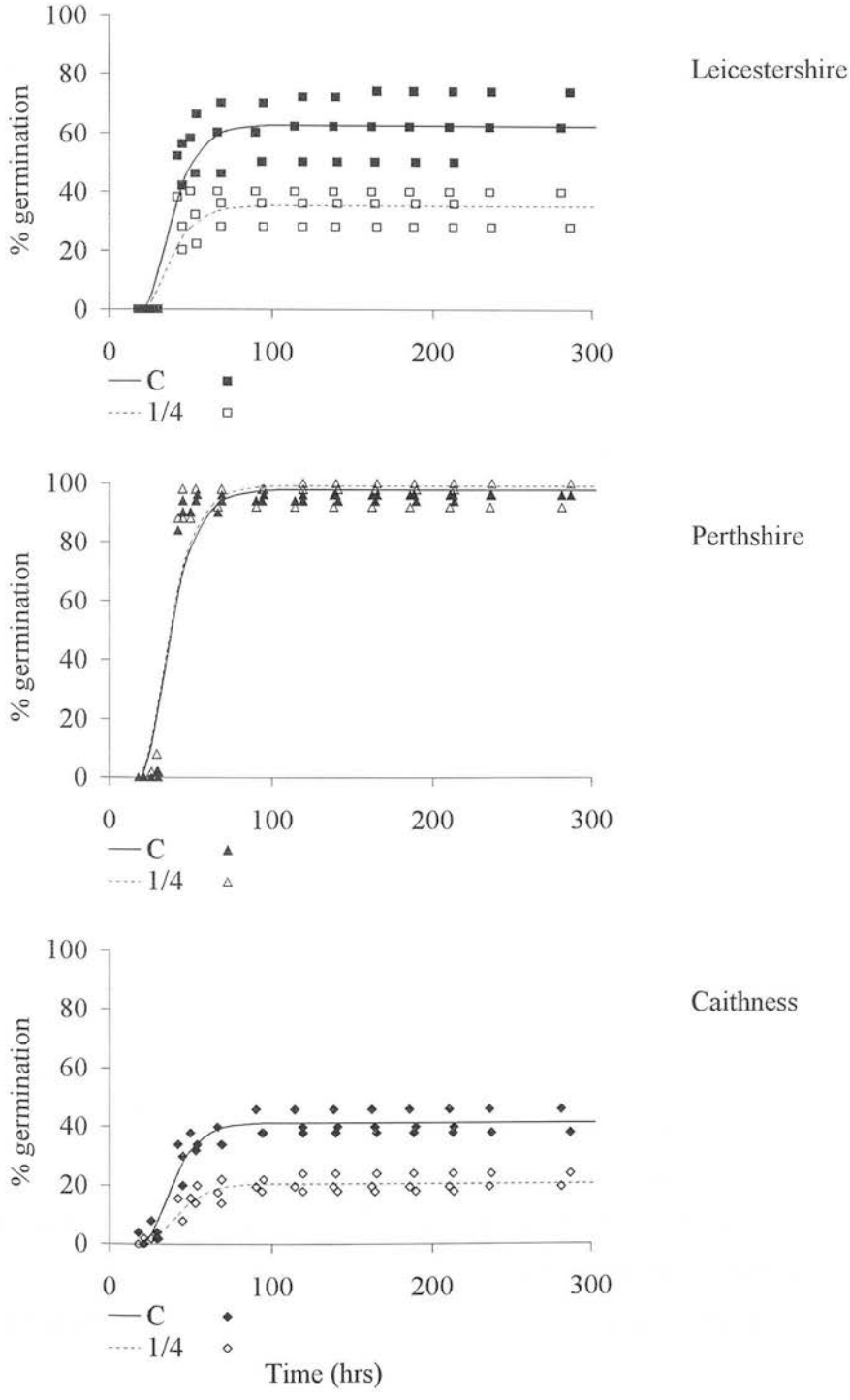


Figure 8.8: Germination time courses at 20 °C for seed produced without herbicide treatment (C) and following application of 1/4 dose fluroxypyr for three populations of *Stellaria media*.
Lines represented fitted curves. n = 3.

8.5. Discussion

8.5.1. Plant growth and seed set following fluroxypyr treatment

Fluroxypyr treatments were applied when the seedlings were at eight to ten leaves, prior to flowering. Overall significantly more seeds were produced in control and very low dose treatments compared to higher dose applications of fluroxypyr. This was consistent with the results of Hald (1993), Rasmussen (1993), Andersson (1994a, b, 1995) and others.

It is evident from Figure 8.1 and Figure 8.2 that the application of fluroxpyr at higher doses reduced plant growth and delayed the onset of flowering in the Caithness and Leicestershire populations. The Perthshire population remained consistently late flowering and first flowered in July. The late flowering in this population was associated with relatively low levels of seed production.

Late flowering may also explain reduced seed production following application of higher doses of fluroxypyr. Low levels of seed production were recorded for plants from the Leicestershire population treated with 1/4 and 1/2 dose fluroxypyr, with most or all seeds produced in September, as opposed to July and August for plants treated with lower doses. For the Caithness population most seed was produced in June and July, regardless of treatment, and this suggested that plants recovered from initial delays in flowering associated with higher dose applications of fluroxypyr. However for both populations, the last plants to be harvested tended to be plants treated with the higher rates of fluroxypyr. This suggests that herbicide application may have prolonged the life of the treated plants and allowed greater than expected seed production. This would have blurred differences in levels of seed production between treatments. Andersson (1994a) notes that recovery of treated plants is a problem in pot experiments and notes that such experiments tend to over-estimate seed production. Certainly plants treated with the lower doses of fluroxypyr would struggle to recover in competition with crop plants for water, nutrients or light and the differences between treatments would be expected to be greater in a field experiment.

The timing and duration of flowering is one factor that may explain differences between populations and treatments in total seed production. Seed production will also be a function of the number of flowers, which in turn may reflect differences in plant biomass (Harper, 1977). Interestingly according to population, above ground vegetative dry weight was proportional to total seed weight, with the highest values recorded for the Perthshire

population and the lowest for the population from Caithness. This represented marked differences between populations in reproductive effort (Harper & Ogden, 1970). It was not clear whether this was a general population characteristic or represented adaptive behaviour, whereby early in the season, plant fitness is maximised by allocating resources to seed production and seed survival, whereas late in the season, plant persistence to set seed in more favourable conditions is advantageous.

Within populations there was a general pattern of lower seed production associated with lower above ground vegetative dry weight, although Figure 8.5 showed that this relationship was poorly defined, especially for the Leicestershire and Caithness populations. This contrasts with significant linear regressions for *Chenopodium album* (Rasmussen, 1993) and *Viola arvensis* (Grundy *et al.*, 1995).

It is possible that this poor correlation between above ground dry weight and total seed weight for the Leicestershire population and relatively poor relationship for the Caithness populations may be an artefact of the need to harvest *S. media* seed at intervals over the growing season. Although efforts were made to maximise the number of seeds collected, it may be that some seeds escaped collection and dispersed between collection times. This was likely to be less of a problem for the Perthshire population because of the more restricted duration of seed set and because from observation, the capsules of the Perthshire population contained fewer seeds and appeared to be more resistant to splitting.

It is clear that there was marked variability in the timing and duration of flowering and seed set between populations and according to herbicide dose. As such and given the variability in environmental conditions during this period, it was sensible to restrict further investigations to seed collected within a specific time interval.

8.5.2. Seed size and weight following fluroxypyr treatment

Herbicide application reduced seed size, as measured by seed diameter for two of the three populations, but similar reductions were not recorded for measurements of seed weight. It is not clear why there was a discrepancy between the two measurements, although greater precision and replication in measurements of seed weight would have increased data reliability. Champion *et al.* (1997, 1998) reported similar reductions in seed size for seed produced by field populations of *Veronica persica* following treatment with fluroxypyr.

Fluroxypyr was applied during seed production and the increased proportion of small seeds was attributed to relatively early senescence, caused by herbicide-related moisture loss (Champion *et al.* 1997, 1998). In this experiment herbicide application preceded seed production and therefore an alternative mechanism is needed. As such, it is suggested that reductions in seed size were associated with plastic behaviour to maintain seed output. Seed output was not reduced following herbicide treatment for Perthshire population and seed size remained constant.

Effects of herbicide treatment on seed size have varied, with some authors similarly reporting reductions in seed size (Andersson, 1996; Champion *et al.*, 1997, 1998), some reporting increased seed size (Hume & Shiriff, 1989, Andersson, 1994b) and others reporting no effect (Grundy *et al.* (1995). These reports have involved different weed species, different herbicides and different timings of application and it is therefore difficult to generalise. It is now also apparent that there can be different responses for different populations of the same species. The consequences for seed germination of these population specific, herbicide-related changes in seed size will now be discussed.

8.5.3. Germination of seed produced following application of fluroxypyr

Final percentage germination was the only germination statistic that was significantly effected by herbicide treatment and then for only the Leicestershire and Caithness populations. Final percentage germination tended to be higher for seeds produced without herbicide treatment or following application of the lowest dose (1/64) of fluroxypyr. Andersson (1996) recorded similar reductions in seed germination for *G. spurium* and *T. arvense* following low dose applications of tribenuron-methyl and for *F. convolvulus* following low dose applications of MCPA and Champion *et al.* (1998) recorded lower seed germination for *Veronica persica* following applications of fluroxypyr.

There are several possible mechanisms by which fluroxypyr application might effect final percentage germination. Firstly, both Champion *et al.*, (1998) for *Veronica persica* and Andersson (1996) for *G. spurium* attributed reduced seed germination directly to herbicide effects on seed size, arguing that smaller seeds had reduced levels of stored seed nutrients and this limited seed germination. This seed size argument has some credence as reduced seed germination was only recorded for the Leicestershire and Caithness populations, the same populations where reduced seed size was recorded following herbicide application. It

is also supported by observations that endosperm varies with seed size (Hodgson & Mackey, 1986).

Secondly, herbicide effects on plant phenology and the timing of seed production may explain these differences, such that seeds are produced under different environmental conditions. This argument is discredited as maternal effects were controlled in this experiment, with germination tests conducted using seeds produced within a limited time frame and under very similar environmental conditions.

Thirdly herbicide chemistry and persistence may explain reduced seed germination. Fluroxypyr is classified as an auxin type herbicide and disrupts cellular growth processes, nitrogen metabolism and enzyme production (Environmental Protection Agency, 1998). It is also known to be rapidly translocated within the plant and persist within treated tissue (Environmental Protection Agency, 1998). Given these chemical effects, persistence and effective translocation, it could be argued that the presence of fluroxypyr in newly produced seeds might explain reduced levels of subsequent seed germination. However, for this experiment this was thought unlikely since herbicide persistence for more than four months would be required to cover the time interval from herbicide application to seed harvest. No data for plant persistence were available, but terrestrial field dissipation of 36.3 days has been recorded (Environmental Protection Agency, 1998) and maximum persistence in soil under UK climatic conditions has been estimated at between 1 and 2 months (Riley & Eagle, 1990). Although not directly comparable, these statistics suggest that plant persistence for more than four months would be improbable.

As such it is suggested that the herbicide mediated changes in seed germination occurred through changes in the distribution of seed sizes, which in turn were related to reductions in above ground dry matter. However populations differed in response and seed size was not necessarily affected by herbicide application. Moreover, there was also no simple relationship between herbicide dose, seed size and seed germination, though higher doses tended to have greater effects.

Analysis of seed collection times showed that the Leicestershire population was more profoundly effected by the higher doses of fluroxypyr than the Caithness population, and took longer to recover, flower and set seed. Regarding the relatively high levels of germination recorded for seeds produced following the 1/2 dose fluroxypyr treatment, this

may then be explained by the fact that these seeds were harvested in September, not between mid July and mid-August as for the other populations and treatments. As such they were produced in differing environmental conditions and maternal effects may explain this anomalous result.

The distinction between all herbicide treated (including 1/64 dose) and untreated seeds in final percentage germination for the Caithness population at 30 °C is also difficult to explain. It is clear from this experiment and others in this study, that germination of the Caithness population was promoted at 30 °C. It has been suggested that this is probably a response to conditions outwith the normal range of temperature experienced by this population and as such, it is possible that the greater stress of these conditions magnified small differences between untreated and seeds produced following very low levels of herbicide application.

The differential response of populations in plant growth following herbicide application and subsequent population x treatment effects on seed production, seed size and seed germination again demonstrate the importance of considering intraspecific variation in studies of weed ecology.

8.6. Conclusions

Populations varied in susceptibility to the herbicide fluroxypyr. Herbicide application affected plant survival and plant growth, with some population-specific mortality recorded following treatment at 8-10 leaves with 1/2 and 1/4 dose fluroxypyr. Significant reductions in seed production were also recorded for two of the three populations treated. For these same two populations, seed size was significantly reduced and this was associated with reduced levels of germination in seed produced following fluroxypyr application. It is suggested that herbicide related effects on subsequent seed germination occurred through changes in the distribution of seed sizes, not through persistent effects of herbicide chemistry. The increased proportion of small seeds following herbicide application was thought to reflect plastic behaviour to maintain seed output, despite reductions in above ground dry matter.

Herbicide application prior to seed production did not affect subsequent germination synchronicity or speed of germination, but significant differences were recorded between

populations. These differences were consistent with other reports in this study (Chapters 2, 6 & 10).

This experiment was done in the absence of competition and with a single timing of herbicide application and it is likely that the results showing decreasing seed production and decreasing plant dry weight with increased herbicide dose would be clearer in the presence of crop competition. It is also clear that applying the herbicide at different stages of weed growth may also have affected both the amount of seed produced and the qualities of that seed, and this requires further investigation.

Chapter 9. The effect of fluroxypyr on seed production and seed germination for contrasting populations of *Galium aparine*.

9.1. Summary

An experiment was conducted to investigate the effect of reduced rate herbicide applications on subsequent seed production and seed germination for three contrasting populations of *Galium aparine*. Reduced rate herbicide applications were selected as an easy method of manipulating plant growth for investigations of variation in seed characteristics, according to differences in the maternal environment. They also represent an integrated approach to weed control in arable cropping. The herbicide used was fluroxypyr and it was applied at 3-4 whorls.

Populations of *G. aparine* differed in their sensitivity to fluroxypyr. Population differences were evident in plant growth, seed production, seed size and seed germination characteristics following low dose fluroxypyr applications.

Herbicide applications markedly affected plant dry weight and the total weight of seeds produced by treated *G. aparine* plants. Both generally decreased as herbicide dose increased, resulting in an approximately linear relationship between seed production and plant size.

For one of the three populations, individual seed weight was reduced by low dose fluroxypyr applications. This population was associated with the most pronounced herbicide-related reductions in seed germination and significant decreases in germination speed. However reduced seed germination following herbicide treatment was also recorded for the other two populations, albeit only when tested at 20 °C and not at 5 and 10 °C and herbicide related reductions in germination speed were recorded for one of these two populations. This suggested that herbicide-related maternal effects on seed germination cannot solely be explained by changes in seed size distribution and may involve herbicide chemistry. It also demonstrates the importance of testing germination in a range of conditions in order to detect subtle changes in seed germination behaviour, which may have important implications for future weed control.

9.2. Introduction

Application of the herbicide fluroxypyr significantly affected seed production and seed germination in populations of *Stellaria media* (Chapter 8). Significant population differences were recorded and herbicide-related reductions in seed germination were restricted to populations where smaller seeds were produced following treatment. This suggested that herbicide-mediated effects on seed germination were related to modifications in seed size, not chemical persistence. This appeared to support similar findings for *Veronica persica* following application of low doses of fluroxypyr (Champion, Froud-Williams & Holland, 1998) and *Galium spurium* following low doses of tribenuron methyl (Andersson, 1996). However Andersson (1996) could not correlate reduced seed size with reduced germination for either *Fallopia convolvulus* or *Thlaspi arvense* following applications of tribenuron-methyl or MCPA. This chapter will apply the same methodology to identify the effect of low dose herbicide applications on seed production, seed size and seed germination for three contrasting populations of *G. aparine*. This includes investigations of the effects of herbicide application on subsequent patterns of germination timing..

It was suspected that there was greater potential for herbicide related effects on subsequent seed production, seed size and germination characteristics in *G. aparine* compared with *S. media*. This was because flowering and seed set tend to occur over a relatively shorter period in *G. aparine* and the seeds produced tend to remain on the parent plant prior to dispersal at the end of the summer (Malik & van den Born, 1988). This would mean that opportunities for plant recovery were more limited and that potentially there was a longer period for plant to seed transfer of residual herbicide. Moreover there was also evidence of herbicide-related reductions in seed size and seed germination in the closely related *G. spurium* (Andersson, 1996). However there were no previous reports of herbicide effects on subsequent germination timing.

The selected herbicide was fluroxypyr (Starane 2: DowAgrosciences). This was for comparison with the study on *S. media* (Chapter 8) and because fluroxypyr is specifically recommended for *G. aparine* control in cereal crops. Fluroxypyr is also widely used and UK farmers often reduce doses below that recommended by the manufacturer in order to reduce costs (Ken Davies, pers. comm.). Fluroxypyr was described in detail in Chapter 8.

Hill & Courtney (1991) reported differences between populations of *G. aparine* for tolerance to fluroxypyr and they suggested that these differences were related to differential rates of metabolic detoxification. It follows that if this is a widespread phenomenon in *G. aparine*, maternal applications of fluroxypyr could produce markedly different levels of seed production and seed germination responses according to population, with important consequences for subsequent weed control.

9.3. Methods

9.3.1. Seed used

The experiment initially used mature seeds that were harvested in August 1996 from three populations of *Galium aparine* (as identified in Chapter 3). The seed was harvested from plants grown in an unheated glasshouse with seed set under muslin to prevent cross-pollination. It should be noted that use of seed replicated in a common environment was to minimise initial differences between populations related to differences in maternal environments. The seeds were stored dry in an incubator maintained at 10 °C (± 2 °C) prior to the experiment.

9.3.2. Herbicide treatment, plant growth and seed production

In November 1997, 150 pots (17.8 cm diameter) were filled with a standard peat and perlite mixture (defined in Appendix 1). For each *G. aparine* population, 50 pots were surface sown with five seeds and the pots were placed, with populations grouped together, in two blocks in an outdoor cage. A distance of approximately 2 m separated the populations within the blocks to minimise cross pollination between populations. Pots were watered regularly and on emergence the seedlings were thinned to a single plant per pot.

On 01 April 1998, plants were moved outside for herbicide treatment. Due to poor establishment, replication was reduced to five established plants per population. The weather was dry, wind speeds were low and air temperature was average (~10 °C). The plants had approximately three to four whorls and no buds or flowers were recorded. Plants were sprayed with one of five treatments: 1/2 dose (100 g a.i. ha^{-1}), 1/4 dose (50 g a.i. ha^{-1}), 1/16 dose (12.5 g a.i. ha^{-1}) or 1/64 dose (3.125 g a.i. ha^{-1}) fluroxypyr (Starane 2: Dow Agrosciences) and a control set was sprayed with an equal volume of tap water. Treatments

were applied by trained staff using a Cooper Pegler CP3 knapsack sprayer with a 1.8 m boom, equipped with 6 Orange Lurmark nozzles (F80/0/80/2 Medium). This was calibrated to produce a medium spray quality at 2.75 bar with a water volume rate of 230 l ha^{-1}.

Seedling survival was recorded and growth patterns were recorded six and sixteen weeks after treatment. As the plants grew, canes and twine were used for support and to restrict interaction between neighbouring pots. Seeds were harvested by hand on senescence of the parent plants in September 1998. Harvested seed was placed in a tray lined with absorbent paper and labelled with population and treatment codes and date of harvest. The seed was then left to dry at room temperature for two weeks. It was then hand cleaned and transferred in labelled, sealed paper envelopes to a cooled incubator maintained at 10 °C (± 2 °C) with silica gel used to maintain low relative humidity.

On harvest, the remaining above ground plant material was harvested and placed in a labelled paper bag. The remaining above ground plant material was dried in an oven at 100 °C for 24 hours and then weighed. After final harvest, cleaned seeds from each pot were weighed separately to estimate the weight of seeds produced per plant.

Differences between populations and treatments for above ground vegetative matter at harvest and total seed weight were assessed by analysis of variance (Genstat 5).

9.3.3. Seed weight

Seeds were bulked together by population and treatment for use in subsequent germination experiments. 15 seeds were randomly selected from each population x herbicide treatment and were individually weighed. Analysis of variance (Genstat 5) was used to identify differences in seed weight between populations and treatments.

9.3.4. Seed germination

Starting in January 1999, the germination characteristics of the seed harvested from the herbicide treated plants were assessed at three constant temperatures (5, 10 and 20 °C) following an initial exposure to red light and subsequent intermittent exposure to very low levels of green light.

For each germination test, 30 seeds were placed on a double layer of filter paper (Whatman no. 181) in a 9 cm plastic Petri-dish. There were four replicates for each population x herbicide x temperature treatment, established in two sets separated by 10 days to counter the lack of replication in incubators. Under a green safe light, each Petri dish received 10 ml of distilled water and was then sealed in a self-seal clear polythene bag to minimise water loss. The green safe light was given by an Osram L 36W/30 fluorescent tube and diffuser covered with two layers of dark yellow-green Cinemoid filter (Lee filters #90), giving a photosynthetically active photon flux density (PPFD) of 0.51 ± 0.19 μmol m^{-2} s^{-1}. Petri-dishes were then exposed to red light for 15 minutes. The red light was given by an Osram L 36W/30 fluorescent tube and diffuser covered with two layers of bright red Cinemoid filter (Lee filters #26), giving a PPFD of 3.84 ± 0.71 μmol m^{-2} s^{-1} (PPFD recorded by quantum sensor and a Campbell CR10 datalogger). The Petri dishes were then randomly placed in cardboard boxes under the green safe light, which were then sealed, wrapped in black polythene and lined with bubble wrap, with a separate box for each temperature. The boxes were then removed to unlit incubators (Gallenkamp/Cryotechnics cooled) maintained at the defined constant temperatures (each ± 2 °C). Germination was assessed under the green safe light, daily for the first ten days and then at less frequent intervals until germination ceased. Seed germination was defined as radicle emergence to greater than 1 mm and germinated seeds were removed from Petri dishes when recorded.

For statistical analysis, a Gompertz curve was fitted to cumulated seed germination data for each Petri dish by least squares (Genstat 5). The Gompertz curve was defined as detailed in Chapter 2.

The Gompertz distribution was also used to estimate time to 50 % germination (t_{50}) for each data set. Analysis of variance (Genstat 5) was used to identify differences in the fitted parameters of the Gompertz curve and estimated t_{50} between populations and between test environments. Data sets that were poorly fitted by the Gompertz curve ($r^2 < 0.9$) were excluded from the analysis.

Additional data on subsequent seedling growth was collected, but these will not be presented.

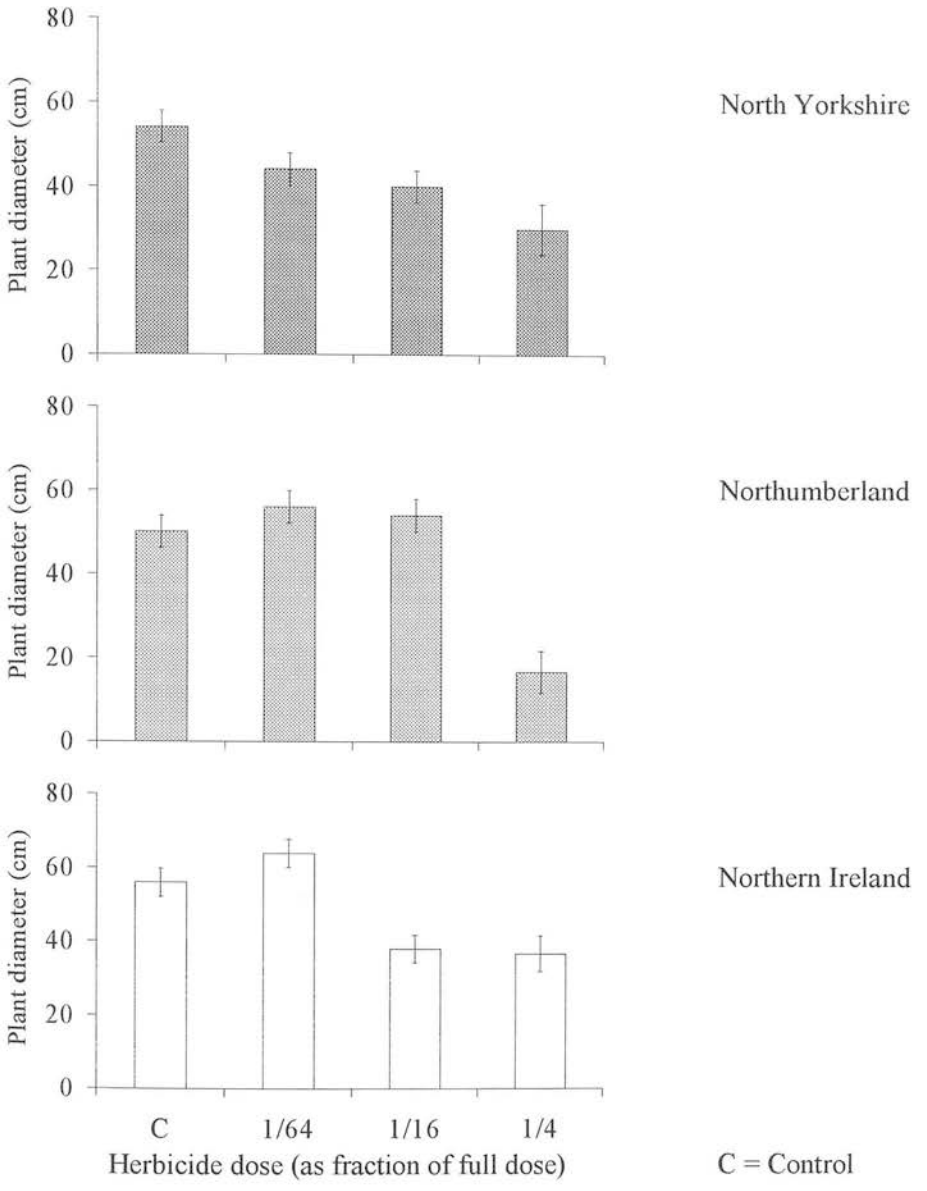
9.4. Results

9.4.1. Plant growth and seed set following fluroxypyr treatment

Following spraying on 01 April 1998, observations 6 weeks later showed that 1/2 dose fluroxypyr killed plants from all three populations. Plants survived application of all lower doses, but application of 1/4 dose fluroxypyr reduced the number of plants that produced seeds from five to three for the Northern Ireland and Northumberland populations and to two for the North Yorkshire populations. Plants treated with 1/4 dose fluroxypyr also exhibited marked die back and characteristic leaf curl and root proliferation. Similar characteristics were observed in plants treated with 1/16 dose fluroxypyr.

Figure 9.1 shows differences in plant size between populations and between treatments 16 weeks after herbicide application. It was clear that application of 1/4 dose fluroxypyr significantly reduced plant diameter compared to the control treatment for each population. However, the populations differed in sensitivity to fluroxypyr, with no differences in plant size recorded in the Northumberland population, except following 1/4 dose fluroxypyr application compared with significant effects of 1/64 dose application for the North Yorkshire population. Figure 9.2 shows that there were also marked differences between populations for the estimated percentage of green tissue remaining. For each population, a significantly greater proportion of tissue remained green following application of 1/4 dose fluroxypyr compared with control and lower dose treatments. It was also clear that populations differed, with proportionally more green tissue remaining for plants from the Northern Ireland population, regardless of treatment.

Figure 9.3 shows significant differences between populations and between treatments for the dry weight of above ground vegetative material at harvest. The dry weight of above ground vegetative material at harvest was greater for the Northumberland population than for the other two populations. For the North Yorkshire and Northern Ireland populations, final dry weight was significantly reduced by application of fluroxypyr, regardless of dose and there was a consistent decline in final dry weight with increasing herbicide dose. However for the Northumberland population final dry weight was not significantly reduced, except following applications of 1/4 dose fluroxypyr.

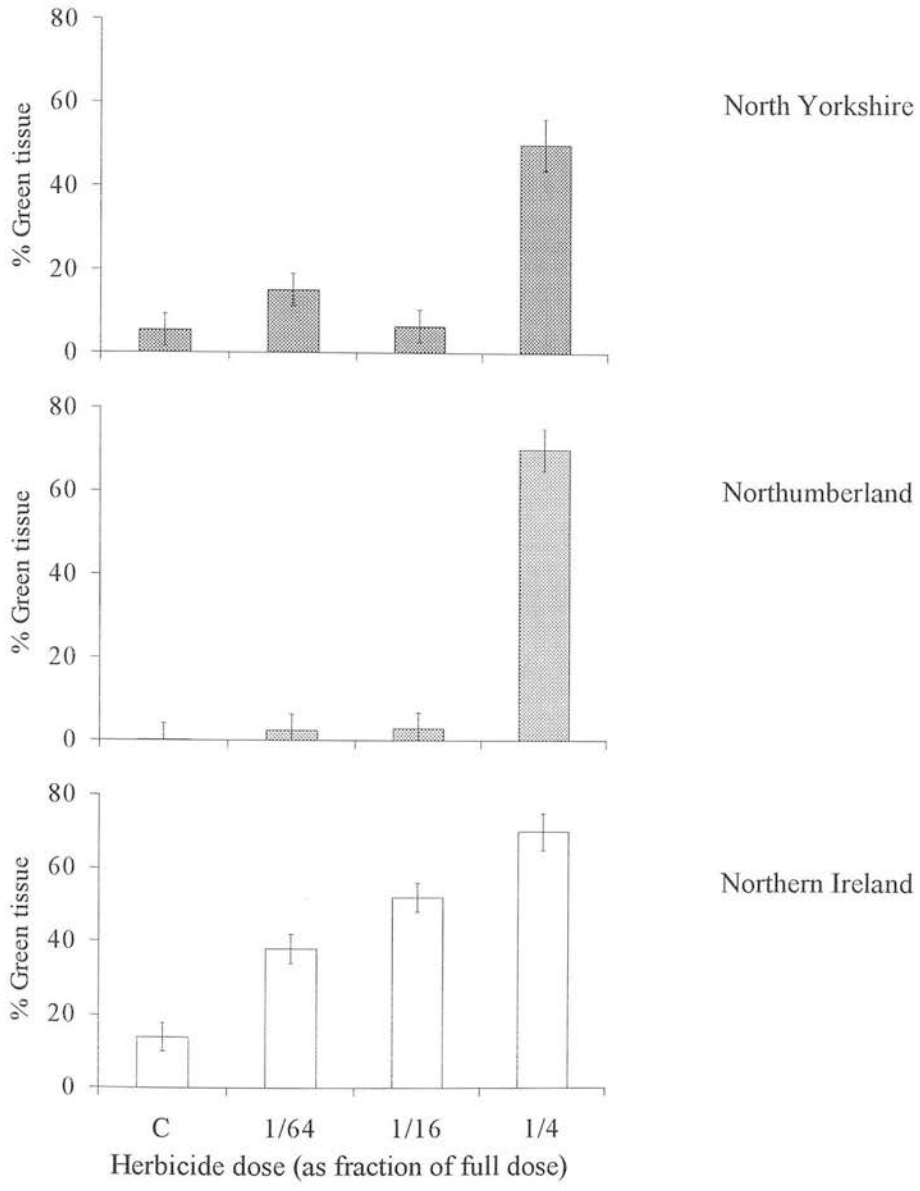


F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	3.22	*
Herbicide dose	3	32.15	***
Population x herbicide dose	6	5.69	***
Residual	41		

(7 missing values)

Figure 9.1: Plant diameter for three populations of *Galium aparine*, 16 weeks after treatment with fluroxypyr at low doses.
Mean plant diameter ± S.E.: C = Control: n = 5.

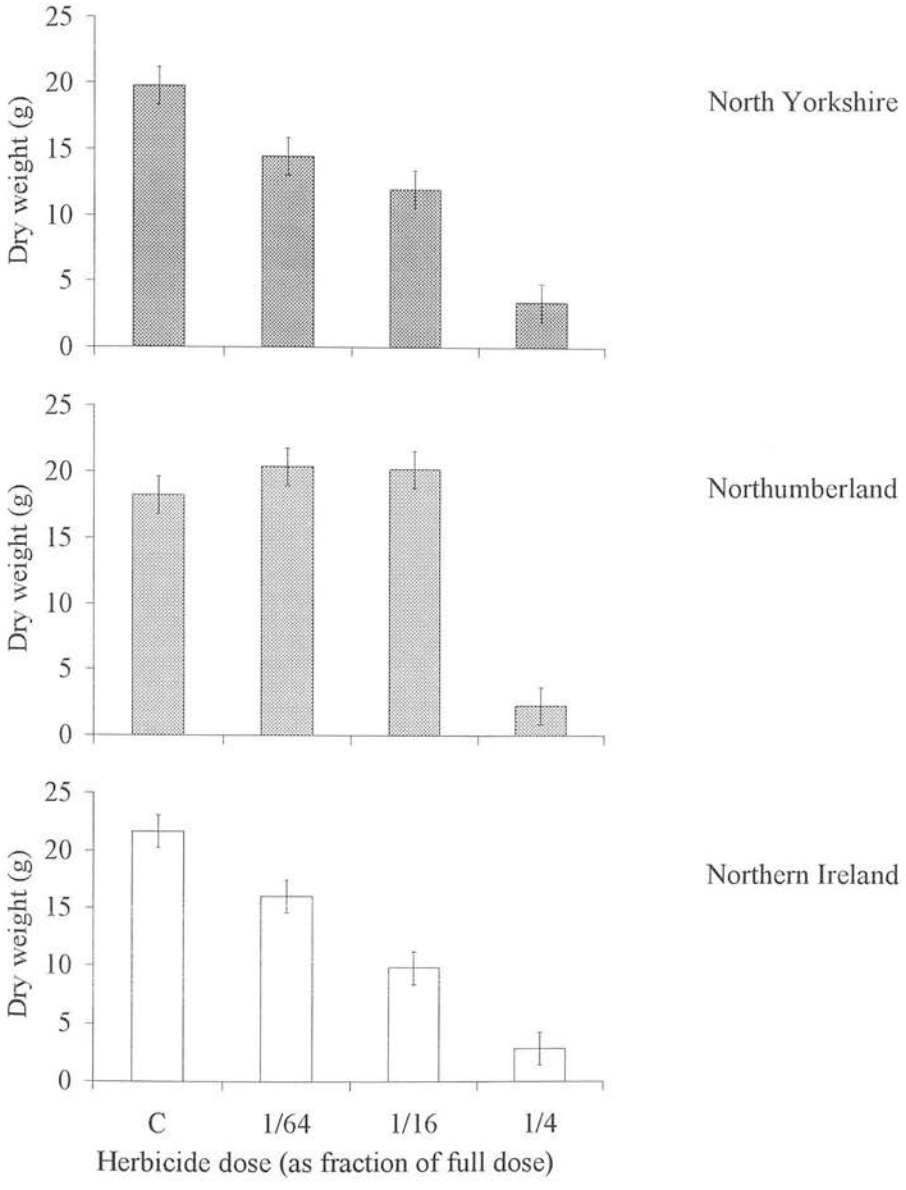


F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	52.72	***
Herbicide dose	3	122.35	***
Population x herbicide dose	6	10.14	***
Residual	41		

(7 missing values)

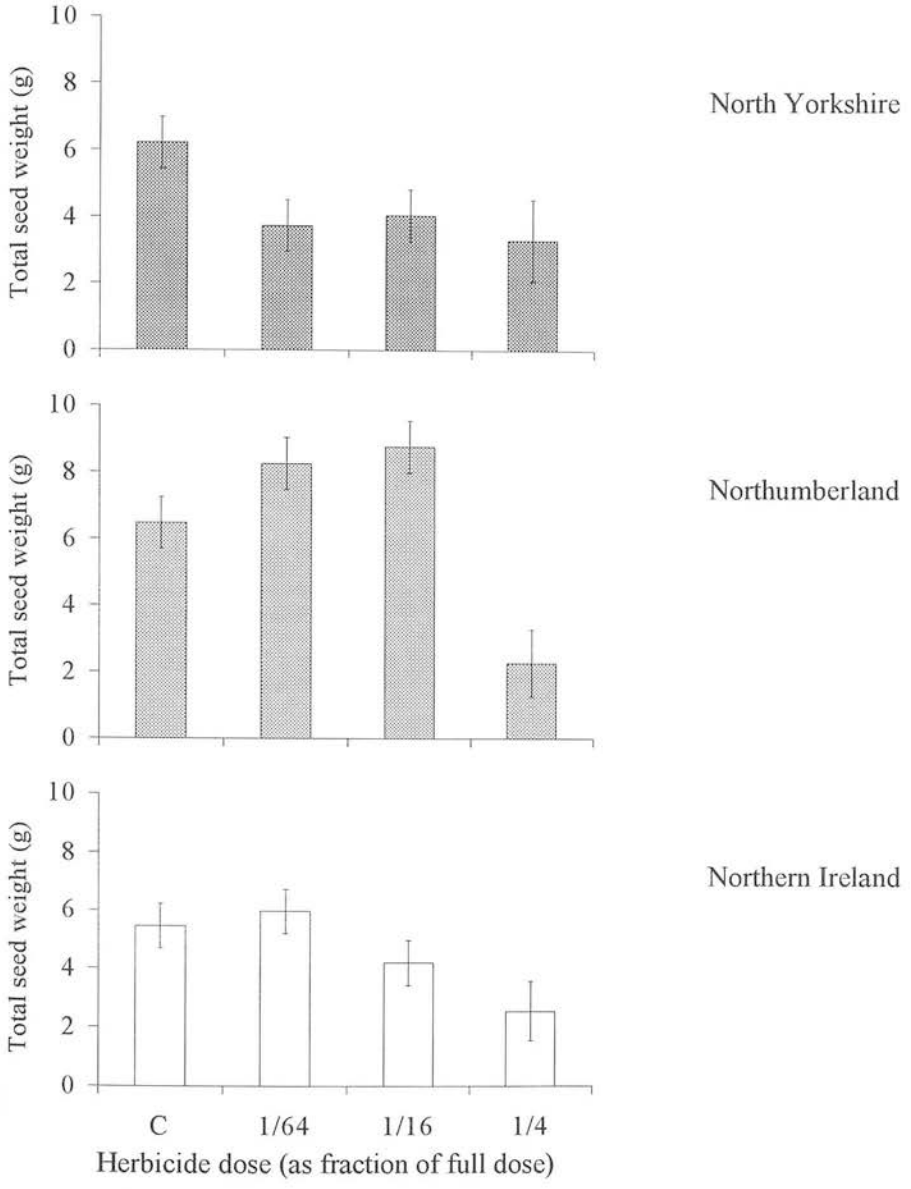
Figure 9.2: Estimated % green tissue for three populations of *Galium aparine*, 16 weeks after treatment with fluroxypyr at low doses.
Mean estimated % green tissue ± S.E.: C = Control: n = 5.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	5.14	**
Herbicide dose	3	83.48	***
Population x herbicide dose	6	5.42	***
Residual	48		

Figure 9.3: Total dry weight of above ground vegetative material after harvest for three populations of *Galium aparine* treated with fluroxypyr at low doses. Mean above ground vegetative dry weight per plant ± S.E.: C = Control: n = 5.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	9.74	***
Herbicide dose	3	13.76	***
Population x herbicide dose	6	4.48	***
Residual	41		

(7 missing values)

Figure 9.4: Total weight of seed produced by individual plants from three populations of *Galium aparine* treated with fluroxypyr at reduced doses.
Mean seed weight per plant ± S.E. : C = Control.

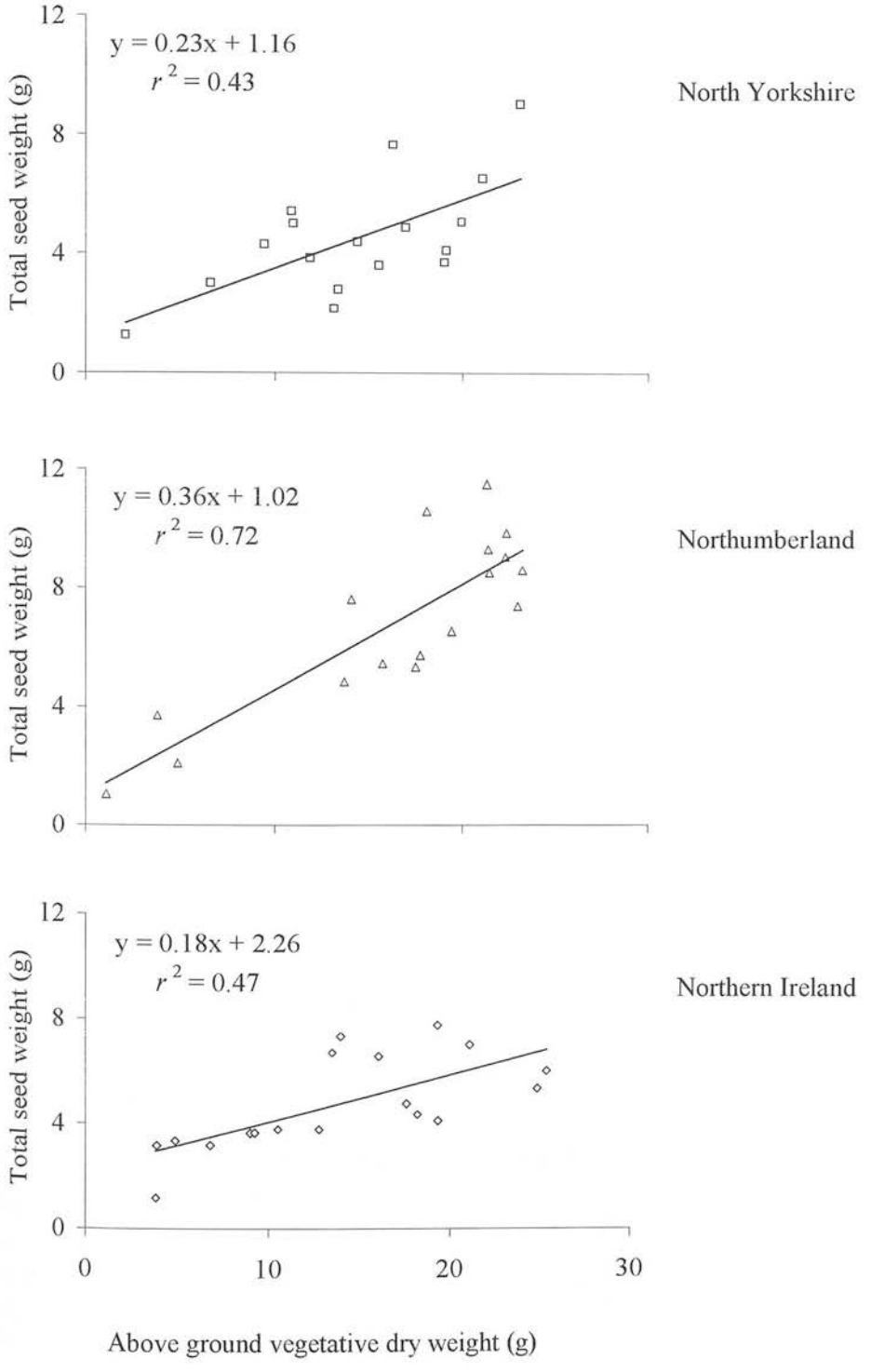
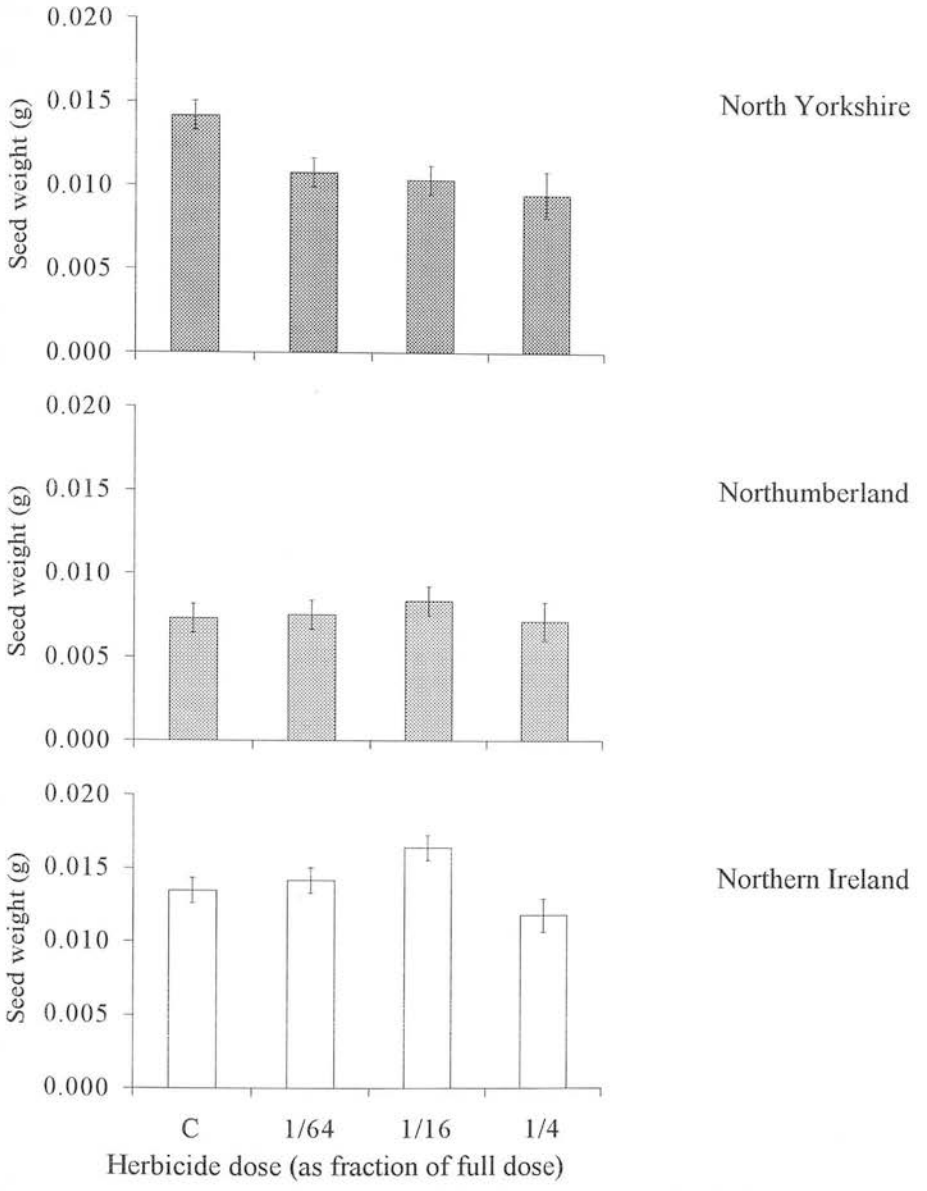


Figure 9.5: Relationship between above ground vegetative dry weight and total seed weight for *Galium aparine* populations.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	54.44	***
Herbicide dose	3	4.27	**
Population x herbicide dose	6	3.27	**
Residual	147		

(21 missing values)

Figure 9.6: Weight of individual seeds produced by plants from three populations of *Galium aparine* treated with fluroxypyr at reduced doses.
Mean individual seed weight ± S.E.: C = Control: n = 15.

Figure 9.4 shows significant differences between populations, between treatments and for the interaction between populations and treatments for the total weight of seed produced by individual plants. The weight of seed produced by the Northumberland population was greater than that produced by the other two populations. Overall the weight of seed produced was significantly reduced by 1/4 dose applications of fluroxypyr. However at lower doses of fluroxypyr application there was variation in the total weight of seeds produced according to population. The Northumberland population produced a significantly higher weight of seeds at 1/16 dose fluroxypyr than in the control, whereas the weight of seeds produced in the North Yorkshire population was significantly reduced by 1/64 dose applications of fluroxypyr. In the Northern Ireland population, total seed weight was not significantly different from the control at doses less than 1/4, although there was a general decrease in total seed weight with increasing dose.

Figure 9.5 shows the relationship between above ground vegetative dry weight and total seed weight. This relationship could be described by linear regression in each case, with the highest gradient for the Northumberland population and the lowest for the Northern Ireland population.

9.4.2. Seed weight following fluroxypyr treatment

Figure 9.6 shows significant differences between populations, between treatments and for the interaction between population and treatment for the individual weight of seeds produced. Seeds from the Northern Ireland population were the heaviest and seeds produced by plant by the Northumberland population were the lightest. Overall, seeds produced without herbicide treatment were significantly heavier than those produced following treatment with 1/4 dose fluroxypyr. However this was only significant for the North Yorkshire population. For the Northumberland population, there was no significant difference in seed weight according to treatment and for the Northern Ireland population, seed produced following treatment with 1/16 dose fluroxypyr was significantly heavier than the control seed and seed produced following 1/4 dose treatment.

9.4.3. Germination of seed produced following fluroxypyr treatment

Table 9.1, Table 9.2 and Table 9.3 give the mean parameters for the Gompertz curves fitted to the cumulative germination time courses for each population x treatment, in each of the three constant temperatures. The significance of differences between populations and

treatments is summarised in Table 9.4. It is important to note that no germination was recorded for seeds of the North Yorkshire population produced after treatment with 1/4 dose fluroxypyr and Gompertz curves could not be fitted to the germination time course of seeds produced by plants that had been treated with 1/16 dose fluroxypyr from the Northumberland population.

9.4.3.1. Differences according to population and test temperature

Significant differences were recorded between populations for the synchronicity of germination, the speed of germination and final percentage germination. The synchronicity of germination was highest for the Northern Ireland population and least for the North Yorkshire population. This mirrored differences in the speed of germination, where germination occurred quickest for the Northern Ireland population and slowest for the North Yorkshire population. The North Yorkshire population had the lowest final percentage germination and the Northumberland population had the highest.

Significant differences were also recorded according to temperature for the synchronicity of germination, the speed of germination and final percentage germination. Germination synchronicity was highest at 10 °C and lowest at 20 °C. Germination speed was fastest at 10 °C and slowest at 5 °C and final percentage germination was significantly less at 20 °C.

There were significant interactions between populations and temperature for the synchronicity and speed of germination and for final germination percentage. The synchronicity of germination of the North Yorkshire population was markedly reduced at 5 °C and that of the Northumberland population was reduced at 20 °C compared to the other populations. These differences corresponded to clear differences between populations for the speed of germination at different temperatures. The North Yorkshire population germinated markedly slower at 5 °C and the Northumberland population was relatively slow at 20 °C. The Northern Ireland population was unique in tending to germinate fastest at 20 °C. Final germination percentage of the Northumberland population tended to 100 % and there were no significant differences according to temperature. For the North Yorkshire population, final germination percentage was significantly higher at 5 compared with 10 °C, which in turn was significantly higher than that at 20 °C. Final germination percentage of the Northern Ireland population was only significantly reduced at 20 °C.

9.4.3.2. Differences according to herbicide treatment

The synchronicity of germination was marginally higher for seeds produced following application of 1/16 dose fluroxypyr only. The speed of germination was significantly reduced for seeds that were produced after application of 1/4 dose fluroxypyr. The final percentage germination was significantly reduced for seeds produced following applications of either 1/16 dose or 1/4 dose fluroxypyr and this is clearly shown in Figure 9.7.

There were no significant interactions between population and treatment for the synchronicity of germination. However for the speed of germination, it was clear that populations differed markedly according to previous treatment. For the North Yorkshire population it was clear that germination was slower than the control for seeds produced following application of either 1/64 or 1/4 dose, but not of 1/16 dose fluroxypyr. For the Northumberland population germination was only significantly slower following application of 1/4 dose fluroxypyr and for the Northern Ireland population there were no significant differences between populations for germination speed according to the different treatments. Final percentage germination was lowest for all three populations for seeds produced following application of 1/4 dose fluroxypyr. However it was clear that the North Yorkshire population was particularly affected by increasing dose rate and final percentage germination following application of 1/4 dose fluroxypyr was much more markedly reduced compared to the other two populations. For the Northumberland population there were no significant differences in final percentage germination between the control and applications of either 1/64 or 1/16 dose and for the Northern Ireland the trend to decreasing final percentage germination with increasing dose was much less marked.

Figure 9.8 contrasts patterns of germination for the seeds produced without herbicide treatment and for seed produced following application of 1/4 dose fluroxypyr.

Treatment	Temperature °C	β hr^{-1}	μ hr	γ	α	$t50$ hr
C	5	0.0175	259.60	86.60	-0.20	285.87
	10	0.0225	186.99	72.31	-0.21	203.97
	20	0.0125	148.17	54.72	-2.54	185.90
1/64	5	0.0175	305.28	87.81	-0.13	328.36
	10	0.0225	228.92	87.19	-0.36	246.04
	20	0.0067	374.94	39.07	-0.49	387.89
1/16	5	0.0175	291.03	60.54	-0.88	318.73
	10	0.0350	183.95	32.60	-0.10	194.77
	20	0.0133	132.26	32.08	-2.33	170.62
1/4	5	0.0167	328.02	18.67	-0.10	352.97
	10	0.0275	298.87	14.47	0.07	317.41
	20	*	*	*	*	*

Table 9.1: Mean Gompertz parameters for *Galium aparine* seeds from the North Yorkshire population produced following fluroxypyr application and germinating at a range of temperatures.
* no germination recorded

Treatment	Temperature °C	β hr^{-1}	μ hr	γ	α	$t50$ hr
C	5	0.0200	239.50	99.90	0.72	260.56
	10	0.0225	182.72	101.54	0.52	199.33
	20	0.0100	248.47	96.30	-0.54	285.53
1/64	5	0.0200	245.01	98.49	0.40	263.05
	10	0.0225	178.86	99.35	0.32	195.44
	20	0.0100	325.72	89.63	4.21	355.57
1/16	5	0.0250	237.36	93.57	0.26	252.53
	10	0.0250	174.63	99.03	-0.27	190.09
	20	*	*	*	*	*
1/4	5	0.0250	287.22	100.20	1.03	301.89
	10	0.0250	214.61	100.95	1.00	229.34
	20	0.0100	418.84	60.59	0.41	454.81

Table 9.2: Mean Gompertz parameters for *Galium aparine* seeds from the Northumberland population produced following fluroxypyr application and germinating at a range of temperatures.
* parameters could not be estimated

Treatment	Temperature °C	β hr^{-1}	μ hr	γ	α	$t50$ hr
C	5	0.0275	270.64	94.89	0.23	285.08
	10	0.0200	184.04	100.09	0.04	202.34
	20	0.0100	151.17	60.25	-2.93	194.99
1/64	5	0.0200	286.34	99.07	0.85	304.03
	10	0.0250	215.66	101.42	1.16	230.27
	20	0.0150	118.75	38.46	-5.04	161.68
1/16	5	0.0325	307.07	98.85	0.09	319.56
	10	0.0325	229.38	98.33	0.39	240.65
	20	0.0200	224.30	17.73	0.23	241.77
1/4	5	0.0250	291.65	92.85	0.14	306.84
	10	0.0250	228.01	100.26	0.44	243.00
	20	0.0100	192.53	19.43	-1.33	238.46

Table 9.3: Mean Gompertz parameters for *Galium aparine* seeds from the Northern Ireland population produced following fluroxypyr application and germinating at a range of temperatures.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	2	6.11	**	6.53	**	76.50	***
Temperature	2	64.18	***	14.74	***	60.78	***
Treatment	3	3.90	*	15.42	***	12.94	***
Population x temperature	4	3.80	**	6.49	***	17.44	***
Population x treatment	6	2.08	-	5.81	***	5.69	***
Temperature x treatment	6	1.12	-	1.68	-	4.17	***
Population x temperature x treatment (1 missing value)	11	1.17	-	1.45	-	2.20	*
Residual	90						

(15 missing values)

Table 9.4: Summary of the analysis of variance for the differences between populations of *Galium aparine* for fitted Gompertz parameters according test temperature and to herbicide treatment.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$

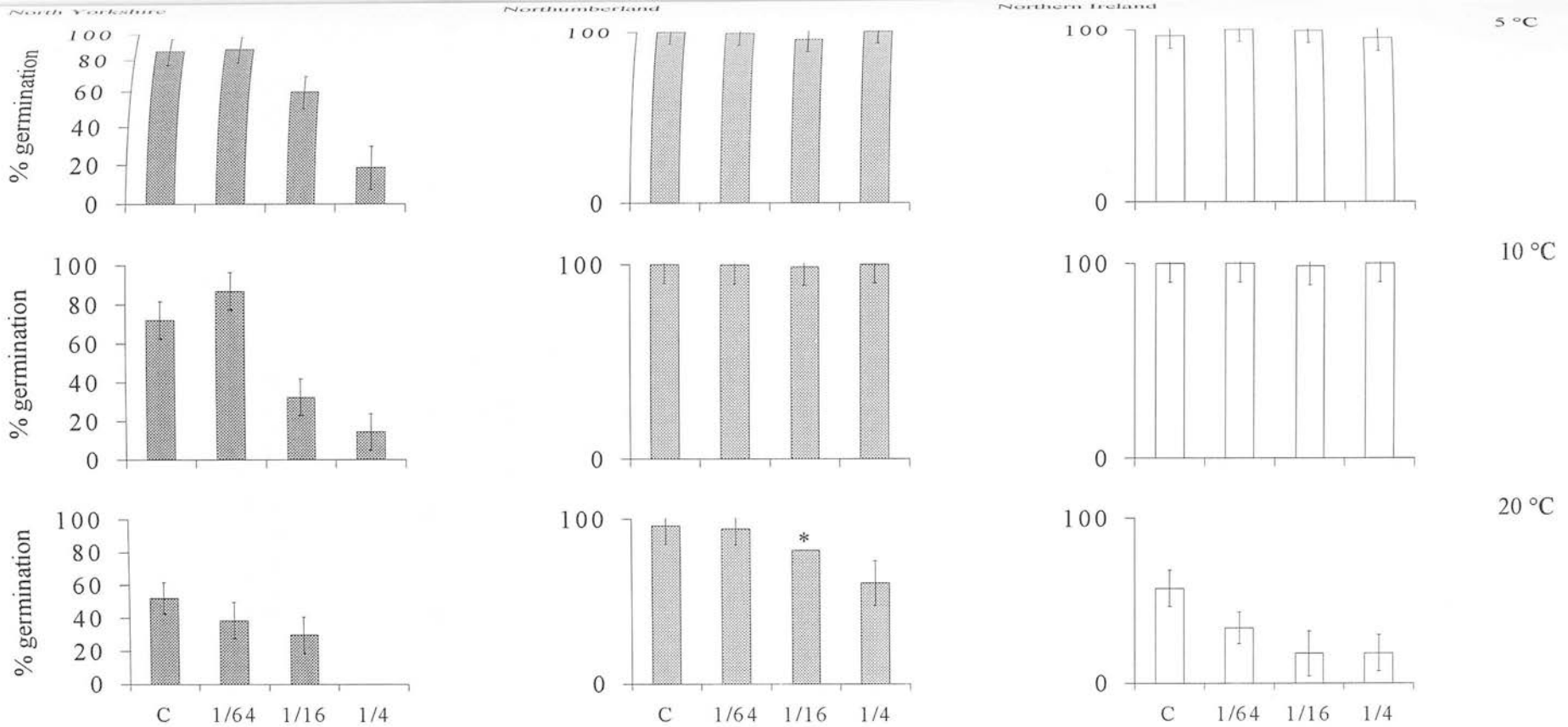


Figure 9.7: Final percentage germination at different temperatures estimated from Gompertz parameters ($\alpha + \gamma$) for *Galium aparine* seeds produced after fluroxypyr application.
Mean ($\alpha + \gamma$) ± S.E.: n = 4: * mean of actual final % germination that could not be estimated from Gompertz parameters.

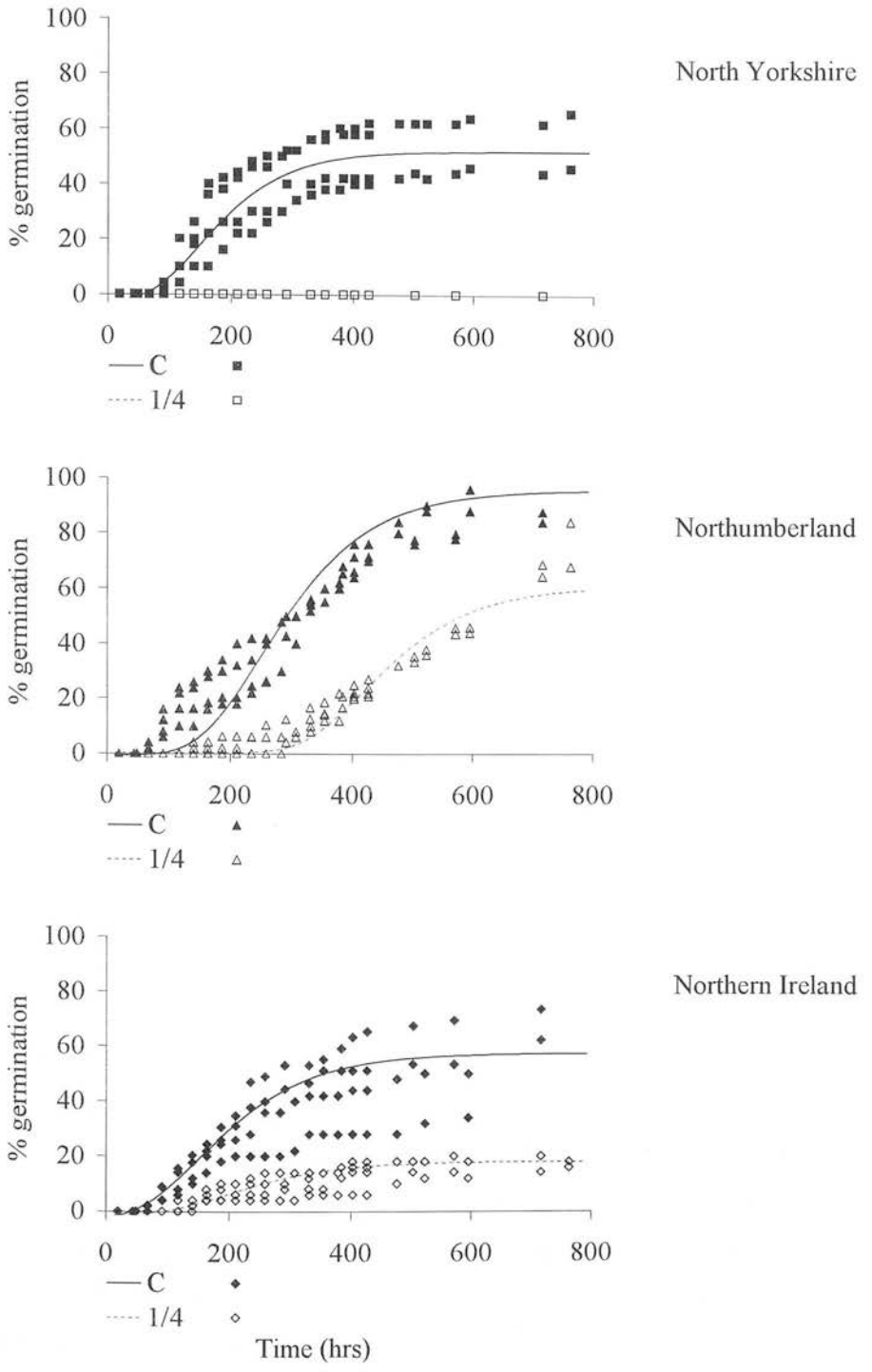


Figure 9.8: Germination time courses at 20 °C for seed produced without herbicide treatment (C) and following application of 1/4 dose fluroxypyr for three populations of *Galium aparine*.
n = 4.

9.5. Discussion

9.5.1. Plant growth and seed set following fluroxypyr treatment

Fluroxypyr treatments were applied when the plants were between three and four whorls, prior to flowering. The half dose killed all plants from the three populations, and suggests that half dose fluroxypyr was more effective at controlling *G. aparine* at three to four whorls compared with *Stellaria media* at eight to ten leaves.

Overall plant size, as measured by both plant diameter sixteen weeks after spraying and by plant above ground dry weight after harvest, was significantly reduced by application of 1/4 dose fluroxypyr in all three populations. It was recorded that populations differed in the effects of lower dose fluroxypyr application on plant size, with the Northumberland population reduced in size only by 1/4 dose fluroxypyr and the North Yorkshire population exhibiting size reductions with each increased dose of fluroxypyr. It is suggested that this reflected differential sensitivity to fluroxypyr, similar to that described by Hill & Courtney (1991) and which Hill, Courtney & Harvey (1996) explained by differential rates of metabolic detoxification. This followed an investigation to establish whether morphological differences between populations might affect spray interception and foliar uptake (Hill, Courtney & Harvey, 1996).

There was a linear relationship between above ground vegetative dry weight and the total weight of seeds produced and this corresponded to previous observations by Hald (1993) and Rasmussen (1993). This relationship showed that higher doses of fluroxypyr application reduced seed production and that this was probably explained by the reduction in above ground vegetative dry weight. However it was noted that extrapolation of these linear relations would predict seed production in the absence of above ground vegetative plant material. This suggests that this relationship is only good for the range of values recorded and that marked non-linearities might be expected at low above ground dry weights. It was noted that for each of the three populations, seed production was recorded for above ground dry weights of less than 5 g. The gradient of the linear relationship showed that seed production in the Northern Ireland population was relatively insensitive to increases in above ground vegetative dry weight compared with the other populations.

9.5.2. Seed weight following fluroxypyr treatment

The Northern Ireland population produced the heaviest individual seeds. It is suggested that the relative insensitivity of seed production in this population to increasing above ground vegetative dry weight might reflect the greater reproductive effort required for producing these larger seeds. In contrast, seed production in the Northumberland population was the most responsive to increases in above ground dry weight. This population also produced the highest total weight of seeds, the lightest individual seeds and seed production was recorded at the lowest above ground dry weight. This suggests that the Northumberland population might be considered to exhibit more ruderal (*sensu*. Grime, Hodgson & Hunt, 1988) characteristics than the Northern Ireland population. As such it is likely that the Northumberland population would be better adapted to continuous cropping (regular disturbance) than the Northern Ireland population. It is interesting to note that continuous cropping is more common in the drier east of the UK compared to the greater frequency of mixed farming in the west (Wiseman, Finch & Samuel, 1993). It is possible that the differences between the two populations might have evolved under the different selection pressures associated with these two farming systems, though smaller seeds would then be expected for the North Yorkshire population.

The effect of fluroxypyr treatment on individual seed weight was only evident for the North Yorkshire population and it was noted that this was the same population that exhibited the most pronounced size reductions and reductions in the total weight of seed produced with increasing herbicide dose.

9.5.3. Germination of seed produced following fluroxypyr treatment

Differences between populations and the interaction between populations x temperature for the synchronicity, speed and final germination percentage were consistent with previous results reported in Chapter 3 and Chapter 7. These differences have been discussed previously and will not be discussed again in this Chapter. Instead the discussion will focus on different patterns of germination for seeds produced following fluroxypyr treatment.

The North Yorkshire population showed the most significant effects of fluroxypyr on the germination of seeds produced by treated plants. Germination speed was higher and final percentage germination was lower at all test temperatures for seeds produced from plants treated with 1/4 dose fluroxypyr. Final percentage germination was also consistently

reduced for seeds produced from plants treated with 1/16 dose fluroxypyr. For the Northumberland and Northern Ireland populations, effects were restricted to seed produced from plants treated with 1/4 dose fluroxypyr. Germination speed was reduced only for the Northumberland population and lower final percentage germination in both populations was evident only when seeds were germinated at 20 °C.

The North Yorkshire population appeared to be the population that was most sensitive to fluroxypyr treatment, as measured by plant size, total seed production and individual seed weight. Gange, Brown & Farmer (1992) speculated that changes in seed size might be one mechanism by which pesticides could affect the germination characteristics of seeds. They suggested that this might occur through changes to the structure and composition of the endosperm, which has been shown to vary with seed size (Hodgson & Mackey, 1986). Similar arguments were used to explain reductions in seed germination associated with smaller seeds following herbicide applications to *Veronica persica* (Champion *et al.*, 1998) and *G. spurium* (Andersson, 1996). It is suggested that changes in seed size (and consequently seed structure) might be one explanation for the more pronounced effects of fluroxypyr application on seed germination for the North Yorkshire population. Certainly it should be noted that there is selective advantage for plasticity in seed size and maximising the number of seeds produced per plant. It could also be argued that there would be further selective advantage from increasing seed dormancy in plants that were produced under stress. However, the precise mechanism by which seed size was reduced and seed germination characteristics changed is not clear. Moreover, it should be noted that the seed size relationship was only true for the North Yorkshire population. Changes to seed germination patterns were also observed for seeds produced by plants that had been treated with 1/4 dose fluroxypyr from the Northumberland and Northern Ireland populations and this was not associated with a decrease in seed size. Instead this could be related to fluroxypyr persistence and incorporation in seeds, changes in cell biology and hormone levels or maternal effects related to seed production under different environmental conditions.

The extent of fluroxypyr persistence in the plant is unclear and as such, this mechanism is speculative, but may merit further investigation in future studies. It is known that fluroxypyr acts by disrupting production of plant auxins and therefore changes in seed size and/or germination behaviour could be a consequence of altered cell biology, though again the particular mechanisms are not known. Although there was no suggestion of differences

between populations in flowering time, it was evident from Figure 9.2 that plants treated with higher doses of fluroxypyr were significantly greener 16 weeks after herbicide application than plants that were untreated or treated with very low doses. This may suggest that flowering was more protracted in these plants and/or that these plants were physiologically different at the time of seed set compared to the other plants that were senescing. As such, this could also explain some of the differences observed.

9.6. Conclusions

Populations of *G. aparine* differed in their sensitivity to fluroxypyr. Whilst 1/2 dose fluroxypyr applications at 3-4 whorls were lethal for all three populations, population differences were evident for plant growth, seed production, seed size and seed germination characteristics in response to lower dose applications.

Herbicide applications markedly affected plant dry weight and the total weight of seeds produced by treated *G. aparine* plants. Both generally decreased as herbicide dose increased, producing an approximately linear relationship between seed production and plant size (Hald 1993; Rasmussen 1993).

For one of the three populations, individual seed weight was reduced by low dose fluroxypyr applications. This population was also associated with the most pronounced herbicide-related reductions in seed germination. This supports work by Champion *et al.* (1998) and Andersson (1996) that linked reduced germination of seed produced following herbicide treatments, to reductions in seed size and associated stored nutrients. Germination timing was also significantly affected by herbicide treatment for this population, with decreased speed of germination recorded for higher rates of fluroxypyr application. However herbicide related reductions in seed germination were also recorded for the other two populations, albeit only when tested at 20 °C and not at 5 and 10 °C and herbicide related effects on germination timing were recorded for one of the two populations. This suggests that, in contrast to the results given in Chapter 8 for *S. media*, herbicide-related maternal effects on seed germination cannot be explained solely by changes in seed size distribution and that alternatively this may involve herbicide chemistry. It also demonstrated the importance of testing germination in a range of conditions in order to detect these subtle changes in seed germination behaviour.

Chapter 10. The effect of nitrogen on seed production and seed germination for contrasting populations of *Stellaria media*.

10.1. Summary

Experiments were conducted to investigate the effect of nitrogen on seed production and seed germination for three contrasting populations of *Stellaria media*. Plant growth and seed production were compared in soils taken from two contrasting stages of an organic rotation, with and without conventional applications of mineral nitrogen fertiliser. Subsequent seed germination and seed germination in response to nitrate or ammonium ion concentration were assessed in laboratory tests. Significant variation was recorded between populations and between treatments.

For all three populations, applications of additional nitrogen increased plant growth and seed production, although the magnitude of increase varied between population and populations exhibited marked differences in phenology and reproductive effort. No significant differences in plant growth and seed production were recorded according to the two soils from different stages in the organic rotation, despite differences in initial soil mineral concentrations.

Significant differences were recorded between populations and soil treatments for seed size and weight. For the Perthshire population there were no significant treatment effects on seed size, but for the Caithness and Leicestershire populations, larger seeds tended to be harvested from plants grown in the more nutrient rich soil and following applications of additional nitrogen.

In terms of plant growth, seed production and seed characteristics, it was shown that the differences attributable to different soil mineral concentrations within the organic rotation were minimal compared to the differences between the organic and conventional farming systems.

Changes in seed size were not associated with subsequent patterns of seed germination and there was a general lack of treatment effects on seed germination for seeds produced in the

different soils, with or without application of additional nitrogen. This suggested that for *S. media* differences between the organic and conventional system may be restricted to differences in seed return, with no immediate effect of soil mineral concentration on patterns of seed dormancy and seed germination.

In laboratory tests, nitrogen addition increased the proportion of seeds germinating and the speed of germination. In laboratory tests, seed germination responded preferentially to nitrogen supplied as the nitrate ion compared to the ammonium ion, although the ammonium ion also promoted seed germination. Germination increased as nitrogen concentrations increased from 1 to 10 mmol l^{-1}, but there was a poor relationship between seed germination and nitrogen concentrations greater than 10 mmol l^{-1}.

The effects of nitrogen on seed germination in the laboratory were not mirrored in the soil experiment where there was no relationship between the number of seedlings that emerged and the different soil and soil nitrogen treatments. This suggests that other factors may limit seed germination in the field.

10.2. Introduction

Nitrogen occurs in soil solution as either nitrate or ammonium ions. Nitrate has been shown to promote germination in a number of weed species (Bewley & Black, 1994) whereas research has tended to show that fewer species germinate in response to ammonium ions (Roberts & Smith, 1977). To date, there has been a sharp divide between controlled experiments looking at the effect of nitrate and ammonium ions on seed germination in the laboratory and extrapolation to germination under field conditions. Nitrate and ammonium concentrations in soils are variable and influenced by many factors including the type of soil, environmental conditions (moisture, temperature) and agricultural practices (Karssen & Hilhorst, 1992). Whereas conventional farming systems tend to fertilise using soluble inorganic forms of nitrate, organic farming systems tend to supply nitrogen as organic matter. Release of ammonium and nitrate ions from organic matter is comparatively slow (Barber, 1984; Haynes, 1986). With increasing interest in organic farming methods, questions have been asked about the effect of changing crop fertilisation practices for weed population dynamics.

Nitrate and ammonium ions are central to the nitrogen cycle (Karssen & Hilhorst, 1992). Nitrogen is fixed by free-living bacteria in the soil and nitrified (NH_4^+ oxidised to NO_3^-) by nitrifying bacteria. Denitrification returns nitrogen to the atmosphere. Seasonal patterns in soil nitrate and ammonium concentrations are difficult to detect, but as the rate of nitrogen mineralisation depends on temperature, higher soil concentrations of nitrate and ammonium ions might be expected in the warmer summer months. However plant growth and uptake often masks this effect and in temperate climates soil nitrate and ammonium levels tend to be highest in late autumn and early spring. In general, soil nitrate levels tend to fluctuate within a range of 5 to 30 mmol l^{-1} (Goudey, Saini & Spencer, 1988).

Nitrate concentrations within the range of 1 to 50 mmol l^{-1} have been shown to promote weed seed germination in experiments (Karssen & Hilhorst, 1992) and in many cases, the stimulative effects of nitrate are enhanced by light and/or alternating temperatures (Vincent & Roberts, 1977; Williams, 1983). Hilhorst (1990 in Karssen & Hilhorst, 1992) has speculated that temperature, light and nitrate interact in the reactions required for germination. The difference between ammonium and nitrate ions has been explained by the assumption that the germination process requires an electron acceptor, although ion size and structure have also been suggested to explain the lower activity of the ammonium ion (Karssen & Hilhorst, 1992). However, whilst Karssen and de Vries (1983) showed that ammonium salts were less active in promoting the germination of *Sisymbrium officinale*, Schimpf and Palmblad (1980) showed that there was no difference between nitrate and ammonium ions in promoting germination of *Amaranthus retroflexus* or *Setaria glauca*. Moreover supra-optimal levels of nitrate have been shown to inhibit seed germination (Karssen & Hilhorst, 1992) and combinations of ammonium and nitrate ions have been shown to be more effective in promoting germination than either factor alone (Karssen & de Vries, 1983; Goudey, Saini & Spencer, 1986). However it should be noted that such experiments have exclusively focussed on germination extent and not the effects of nitrogen on germination timing, despite the importance of germination timing in determining individual growth and development and the outcome of competition (Benjamin, 1990; Ghersa & Holt, 1995; Fenner, 1995). As such, it is apparent that the concentration of nitrate ions and role of ammonium ions in timing and extent of weed seed germination still requires further investigation. Consequently, the first experiment in this chapter is designed to investigate the effect of nitrogen source and concentration on seed germination for three contrasting populations of *Stellaria media*.

The effects of nitrogen on seed germination are further complicated by research that has shown a positive relationship between seed nitrate content and germination in water (Fawcett & Slife, 1978b; Saini, Bassi & Spencer, 1985a, b; Bouwmeester, 1990 in Karssen & Hilhorst, 1992). The nitrate content of seeds on the parent plant have been shown to relate to soil nitrate levels for some species (Saini *et al.*, 1985a, b) and this would suggest that high levels of soil nitrates might promote higher levels of germination in the subsequent seed generation. As it is suspected that high concentrations of soil nitrates would also increase seed production as a consequence of increased plant growth, this allied with effects on subsequent seed germination could result in marked changes to weed population dynamics.

Previous research has shown that germination of dry stored *S. media* seeds was higher in 20 mmol l^{-1} potassium nitrate solution than in distilled water, but germination of freshly harvested seeds, or seeds that were previously buried, was not promoted by 20 mmol l^{-1} potassium nitrate, except in alternating temperatures (Roberts & Lockett, 1975). This demonstrates an interaction of nitrate with storage and temperature conditions, suggesting that the involvement of nitrate in *S. media* germination is complex.

Whilst this complexity was recognised, the aim of this Chapter was to establish the extent of differences in germination response to nitrogen between the contrasting populations of *S. media* identified in Chapter 2. The experiment was designed in two parts. The first part aimed to investigate seed germination responses to difference sources of nitrogen in simple Petri-dish experiments. The second part investigated differences in seed germination for seeds produced from plants grown in soils of different nitrogen status. Additional information on seedling growth characteristics was collected, but these will not be presented.

10.3. Methods

10.3.1. Effect of nitrogen and nitrogen source on seed germination

10.3.1.1. Seed used

The experiment used mature seed harvested in August 1996 from three populations of *S. media* (as identified in Chapter 2). The seed was harvested from plants grown in an unheated glasshouse with seed set under muslin to prevent cross-pollination. It should be noted that use of seed replicated in a common environment was to minimise initial

differences between populations related to differences in maternal environments. Seeds were stored dry in an incubator maintained at 10 °C (± 2 °C) prior to the experiment.

10.3.1.2. Experimental design

Experiments to investigate the germination response of seed to nitrogen according to whether nitrogen was supplied as nitrate or ammonium were started in December 1997. For each population and each treatment, four replicates of 50 seeds were used. The eight treatments are listed in Table 10.1. Replicates were started at approximately 10 day intervals to counter the lack of replication in the temperature environment.

Concentration	H_2O	KNO_3	NH_4Cl	NH_4NO_3
0.0 mmol l^{-1}	X	-	-	-
1.0 mmol l^{-1}	-	x	x	-
10.0 mmol l^{-1}	-	x	x	x
100.0 mmol l^{-1}	-	x	x	-

Table 10.1: Solutions used to investigate the effect of nitrogen and nitrogen source on *Stellaria media* seed germination

Each replicate 50 seed sample was placed in a labelled 9 cm Petri-dish lined with two layers of 9 cm diameter filter paper (Whatman no. 181). Under a green 'safe' light, 10 ml of distilled water or salt solution was added and the Petri-dishes were then sealed in clear polyethylene bags to minimise water loss. The green safe light was given by an Osram L 36W/30 fluorescent tube and diffuser covered with two layers of dark yellow-green Cinemoid filter (Lee filters #90), giving a photosynthetically active photon flux density (PPFD) of 0.51 ± 0.19 μmol $m^{-2} s^{-1}$. The imbibed seeds were then exposed to 15 minutes of red light. The red light was given by an Osram L 36W/30 fluorescent tube and diffuser of bright red Cinemoid filter (Lee filters #26), giving a PPFD of

3.84 ± 0.71 μmol $m^{-2} s^{-1}$ (PPFD recorded by quantum sensor and a Campbell CR10 datalogger). The Petri dishes were randomly placed in a cardboard box under a green safe light which was then sealed. The box was wrapped in black polythene and lined with bubble wrap and then removed to an unlit incubator (Gallenkamp cooled) maintained at 20 ± 2 °C. Germination was recorded after 24, 36 and 48 hrs and thereafter at regular intervals until germination ceased. Germinated seeds (radicle emergence > 1mm) were removed on counting.

For statistical analysis, a Gompertz curve was fitted to cumulated seed germination counts for each Petri dish by least squares (Genstat 5). The Gompertz curve was defined as described in Chapter 2. The Gompertz distribution was also used to estimate time to 50 % germination (t_{50}) for each data set. Analysis of variance with orthogonal contrasts was used to identify differences between populations and treatments for the fitted parameters of the Gompertz curve and estimated t_{50}. Genstat 5 was used for all statistical analyses and data sets that were poorly fitted by the Gompertz curve ($r^2 < 0.9$) were excluded.

10.3.2. Effect of soil nitrogen on seed production, seed germination and seedling establishment

10.3.2.1. Selection of soils

The experiment used soil collected in August 1997 from three neighbouring fields on the SAC Organic Farming Unit at Aldroughty Farm, near Elgin. The fields were at different points in a six-part organic rotation (three years grass and clover, followed by a cereal crop, then a root crop and then an undersown cereal). Table 10.2 lists the field names and differences in crop history for the three sampled fields.

Approximately 250 l of soil was collected from each field from a minimum of ten points within an area of 50 m x 50 m. Soil was collected to a depth of approximately 25 cm. The area from which the soils were collected was located to minimise the distance between fields to approximately 200 metres. Efforts to minimise the distance between fields were intended to restrict variation in soil type. Sub samples of the selected soils were characterised for soil particle distribution, pH, extractable P, K, Mg, organic matter (loss on ignition), total nitrogen and soil mineral NO_3 and NH_4 by SAC Central Analytical Laboratory. Analysis of variance (Minitab) was used to assess differences in soil characteristics between fields. Principal components analysis (Minitab) was then used to identify the two most dissimilar soils and these were then selected to compare the characteristics of seed produced from plants grown in the contrasting soils.

Field	Stage of rotation	Crop
29	1	Perennial rye-grass and white clover
21	5	Potatoes
30	6	Oats undersown with perennial rye-grass and white clover.

Table 10.2: Field names and crop at time of soil sampling, August 1997

Soils from the selected fields were autoclaved to kill the existing soil seedbank and prevent seed contamination (in approximately 10 l sub-samples, for 1 hour at 121 °C). The autoclaved soil was then placed in 17.8 cm diameter pots on top of a 2 cm layer of autoclaved gravel and a thin layer of approximately 500 g of finely sieved soil from the same field that had not been autoclaved. This non-autoclaved soil was included because there was concern that autoclaving would also kill the soil microfauna, which have an important role in nutrient cycling within the soils (Haynes, 1986). The 500 g of soil was intended to reinoculate the soils and restore the original soil microfauna. In order to allow time for this reinoculaton to occur and to check that autoclaving had successfully eliminated the existing soil seedbank, the soils were left in pots in an unheated greenhouse overwinter.

10.3.2.2. Initial seed used

The overwintered pots were sown in May 1998, using seed replicated in common environment and harvested in 1996 (details in section 2.3.2).

Seeds were stored dry in an incubator maintained at 10 °C prior to the experiment.

10.3.2.3. Experimental design

The experiment was designed to assess seed production, seed germination and seedling establishment characteristics for seeds produced by plants grown in two contrasting soils, with and without additional mineral nitrogen. For each population, five replicates per treatment were established in May 1998. For each replicate, five seeds of the named population of *S. media* were surface sown into each pot. For pots treated with additional mineral nitrogen, the equivalent of 100 kg ha^{-1} ammonium nitrate was applied as a finely ground powder to the soil surface at sowing (NitramExtra: 34.5 % NH_4NO_3). The sown pots were placed with populations grouped together in two blocks in an unheated greenhouse. A distance of approximately 2 m separated the populations within blocks and this was intended

to minimise the potential for cross pollination between populations. Throughout the experiment the soils were watered daily in order to maintain soil water content at approximately field capacity. This was important as short term moisture fluctuations can influence nitrate concentrations (Haynes, 1986).

The number of emerged seedlings per pot was recorded and on emergence the seedlings were thinned to a single plant per pot. Characteristics of initial seedling growth and time to flowering were also noted.

Seeds were hand harvested at approximately monthly intervals after initiation of flowering. Seeds were collected at maturity, just prior to capsule splitting and seed dispersal. Harvested seeds were placed in a tray lined with absorbent paper and labelled with population and treatment codes and date of harvest. The seed was then left to dry at room temperature for c. 2 weeks. It was then hand cleaned (using graded sieves) and transferred in labelled, sealed paper envelopes to a cooled incubator maintained at 10 °C with silica gel used to maintain low relative humidity. Cleaned seeds from each harvest were weighed separately and the total seed weight of seeds harvested per plant was calculated.

On the last harvest date (when the plants had senesced), the remaining above ground plant material was harvested and placed in a labelled paper bag. This was then dried in an oven at 100 °C for 24 hours and then weighed.

Differences in plant growth and seed production between populations and between treatments were analysed by analysis of variance (Genstat 5). Analysis of variance for total seed production and correlation between above ground vegetative dry weight and total seed production were restricted to individual plants that produced seed.

Seeds harvested within four weeks between mid July and mid August were bulked together by population and treatment for use in subsequent assessments of seed size, weight and germination characteristics.

Seed size and weight characteristics were determined from these samples. Seed diameter was assessed using callipers to measure individual seed diameter to the nearest 0.05 mm. There were 10 replicates for each population x soil x soil nitrogen treatment. Seed weight was assessed by weighing 5 seed samples, with three replicates for each population x soil x

soil nitrogen treatment. Analysis of variance (Genstat 5) was used to identify differences in seed diameters and 5 seed weight between populations and treatments.

10.3.2.4. Seed germination

The germination characteristics of the seed harvested from the plants grown in different soils, with or without additional mineral nitrogen were assessed starting in May 1999. The assessments were made at four constant temperatures (5, 10, 20 and 30 °C) in either distilled water or 10 mmol l^{-1} KNO_3 solution, each following an initial exposure to red light and subsequent intermittent exposure to very low levels of green light.

For each germination test, 30 seeds were placed on a double layer of Whatman no. 181 paper in a 9 cm plastic Petri-dish. There were three replicates for each population x temperature x soil x soil nitrogen x test nitrogen treatment, established at approximately 10 day intervals to counter the lack of replication in incubators. Under a green safe light (as defined in 10.3.1.2) each Petri dish received 10 ml of distilled water or 10 mmol l^{-1} KNO_3 solution and was then sealed in a self-seal clear polythene bag to minimise changes in water loss through evaporation. Petri-dishes were then exposed to red light for 15 minutes. The red light was defined in section 10.3.1.2. Then under the green safe light, the Petri dishes were randomly placed in sealed cardboard boxes wrapped in black polythene and lined with bubble wrap, with a separate box for each temperature. The boxes were then removed to unlit incubators (Gallenkamp/Cryotechnics cooled) maintained at the defined constant temperatures (± 2 °C). Germination was assessed under the green safe light, daily for the first ten days and then at less frequent intervals until germination ceased. Seed germination was defined as radicle emergence to greater than 1 mm and germinated seeds were removed from Petri dishes when recorded.

For statistical analysis, a Gompertz curve was fitted to cumulated seed germination data for each Petri dish (as described in Section 10.3.1.2). Analysis of variance (restricted to three-way interactions) was then used to identify differences in the fitted parameters of the Gompertz curve and estimated t_{50} between populations and between maternal and test environments. Genstat 5 was used for all statistical analyses and data sets that were poorly fitted by the Gompertz curve ($r^2 < 0.9$) were excluded.

10.4. Results

10.4.1. Effect of nitrogen and nitrogen source on seed germination

Gompertz curves were successfully fitted to each of the cumulated germination time courses for seeds germinated in distilled water or in different nitrogen solutions ($r^2 > 0.9$). The mean values for fitted Gompertz parameters according to population and treatment are given in Table 10.4. Table 10.3 shows that there were significant differences between populations for the synchronicity of germination (β), time to the inflexion point on the fitted Gompertz curve (μ) and final percentage germination ($\alpha + \gamma$). There were also significant differences between treatments for μ and ($\alpha + \gamma$). Significant interactions between populations and treatment were restricted to ($\alpha + \gamma$).

The population from Caithness was consistently less synchronous and germinated more slowly than the populations from Leicestershire and Perthshire. The population from Perthshire germinated to a high level, regardless of treatment. In contrast, germination of the Caithness population was consistently low.

Nitrogen did not affect the synchronicity of germination, but the presence of nitrogen significantly increased the speed of germination and final germination percentage. Orthogonal contrasts applied to the analysis of variance showed that the speed of germination was faster and final germination percentage was higher with nitrogen supplied at concentrations greater than 1 mmol l^{-1}, but that there was no significant difference between concentrations of 10 and 100 mmol l^{-1}. The final germination percentage was also significantly affected by the source of nitrogen, with higher germination recorded with nitrogen supplied as the nitrate ion compared with the ammonium ion. The differences in final germination percentage between populations and treatments are shown in Figure 10.1.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	2	16.87	***	62.27	***	182.48	***
Treatment	7	1.75	-	26.05	***	10.88	***
Population x treatment	14	1.24	-	1.44	-	2.28	*
Residual	46						

Table 10.3: Summary of analysis of variance for differences between populations of *Stellaria media* in fitted Gompertz parameters according to test solution.
F ratios with significance as *** P < 0.001; ** P < 0.010 and * P < 0.050

Population	Solution	β hr^{-1}	μ hr	γ	α	$t50$ hr
Leicestershire	Distilled water	0.1667	44.29	54.82	-0.07	46.50
	1 mmol l^{-1} KNO_3	0.1700	45.65	64.85	-0.09	47.83
	10 mmol l^{-1} KNO_3	0.1700	46.97	80.46	-0.08	49.14
	100 mmol l^{-1} KNO_3	0.1667	53.81	82.59	-0.06	56.03
	1 mmol l^{-1} NH_4Cl	0.1633	43.16	54.34	0.00	45.40
	10 mmol l^{-1} NH_4Cl	0.1633	44.93	66.32	-0.17	47.20
	100 mmol l^{-1} NH_4Cl	0.1600	52.80	65.13	0.19	55.10
	10 mmol l^{-1} NH_4NO_3	0.1167	55.84	85.00	-0.04	58.99
Population means		0.1596	48.43	69.19	-0.04	50.77
Perthshire	Distilled water	0.1700	45.57	94.53	0.29	47.70
	1 mmol l^{-1} KNO_3	0.1700	45.23	93.58	-0.08	47.39
	10 mmol l^{-1} KNO_3	0.1667	44.42	100.17	-0.05	46.63
	100 mmol l^{-1} KNO_3	0.1600	50.39	99.00	0.13	52.71
	1 mmol l^{-1} NH_4Cl	0.1667	45.06	95.07	0.28	47.24
	10 mmol l^{-1} NH_4Cl	0.1733	46.26	95.26	-0.13	48.39
	100 mmol l^{-1} NH_4Cl	0.1533	53.02	96.25	0.89	55.51
	10 mmol l^{-1} NH_4NO_3	0.1700	52.64	96.30	0.78	54.82
Population means		0.1663	47.82	96.27	0.26	50.05
Caithness	Distilled water	0.1533	48.91	26.94	0.33	51.12
	1 mmol l^{-1} KNO_3	0.1133	51.57	47.50	0.30	54.75
	10 mmol l^{-1} KNO_3	0.1667	49.79	62.06	-0.23	52.08
	100 mmol l^{-1} KNO_3	0.1000	60.23	71.37	-0.21	64.41
	1 mmol l^{-1} NH_4Cl	0.0600	54.84	39.09	-0.77	62.85
	10 mmol l^{-1} NH_4Cl	0.1300	54.57	46.82	-0.16	58.16
	100 mmol l^{-1} NH_4Cl	0.1167	59.35	58.19	-0.20	63.25
	10 mmol l^{-1} NH_4NO_3	0.1000	64.00	59.45	0.09	68.11
Population means		0.1175	55.41	51.43	-0.11	59.34
Treatment means	Distilled water	0.1633	46.25	58.76	0.18	48.44
	1 mmol l^{-1} KNO_3	0.1511	47.49	68.65	0.04	49.99
	10 mmol l^{-1} KNO_3	0.1678	47.06	80.90	-0.12	49.28
	100 mmol l^{-1} KNO_3	0.1422	54.81	84.32	-0.04	57.72
	1 mmol l-1 NH_4Cl	0.1300	47.68	62.84	-0.16	51.83
	10 mmol l^{-1} NH_4Cl	0.1556	48.59	69.47	-0.15	51.25
	100 mmol l^{-1} NH_4Cl	0.1433	55.06	73.19	0.29	57.95
	10 mmol l^{-1} NH_4NO_3	0.1289	57.49	80.25	0.28	60.64

Table 10.4: Fitted Gompertz parameters for germination of three populations of *Stellaria media*, in different solutions at 20 °C.

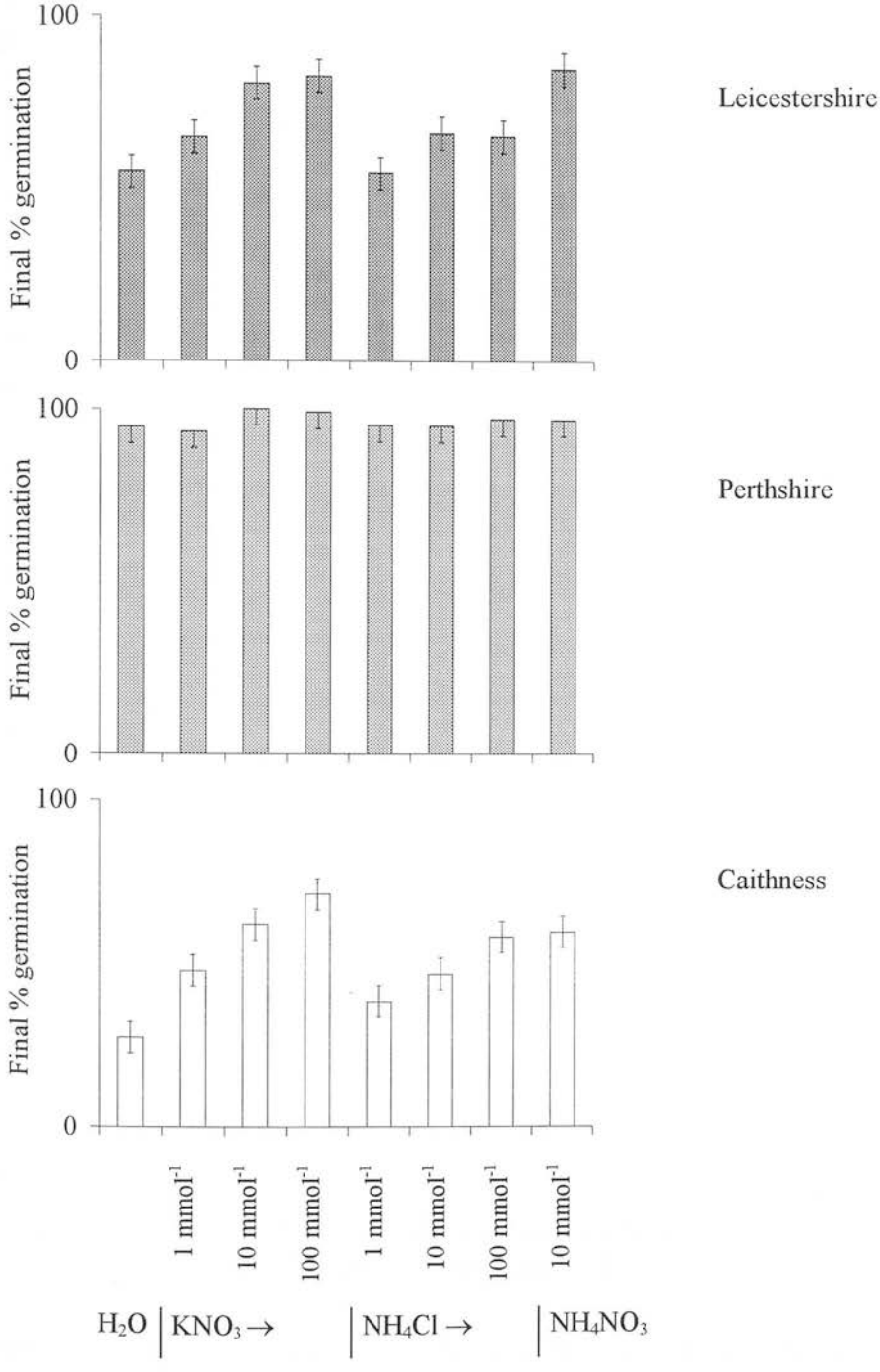


Figure 10.1: Final germination percentage as estimated from Gompertz parameters ($\beta + \alpha$) for different populations of *Stellaria media* in water and different nitrogenous solutions at 20 °C.
Mean final germination percentage ± S.E.: n = 3.

10.4.2. Effect of soil nitrogen on seed production, seed germination and seedling establishment

10.4.2.1. Selection of soils

Table 10.5 illustrates the similarities in physical soil structure for the three sampled fields. The soils were sandy loams, with a high proportion of fine sand. Table 10.6 shows that the soils also had a relatively high organic matter and total nitrogen, as would be expected for fields farmed organically. A significantly higher % organic matter was recorded in field 30 compared with field 29.

Field	Stage	Sand 2 - 0.6 mm	Sand 0.6 - 0.2 mm	Sand 0.2 - 0.06 mm	Course silt	Medium silt	Fine silt	Clay
29	1	5.5	25.3	41.2	14.4	4.3	5.7	3.5
21	5	11.0	30.4	37.2	13.9	3.9	1.8	1.8
30	6	7.6	26.2	39.6	16.9	3.8	3.3	2.7

Table 10.5: Mechanical analysis of soil mineral fraction (< 2.0 mm).
n = 1.

Field	Stage	Crop	Organic matter (loss on ignition) %	Total N mg kg^{-1}
29	1	Grass	7.1	2250
21	5	Potatoes	7.4	2290
30	6	Undersown oats	7.7	2407
		F (2, 6 df)	10.1	3.86
		P	*	-

Table 10.6: Summary of % organic matter and total nitrogen, August 1997.
n = 3: *** P < 0.001, ** P < 0.010 and * P < 0.050.

Table 10.7 summarises the differences between fields for pH and extractable mineral contents. Soils differed significantly regarding pH and extractable levels of phosphorus, potassium, magnesium and nitrate. Field 21 had the lowest pH, lowest levels of phosphorus, potassium and magnesium, whilst field 30 had the highest pH and highest levels of the same minerals, together with the highest level of nitrate. Figure 10.2 shows the plot of principal component scores for each of three replicates for each field. The first axis separated field 30 from fields 21 and 29, whilst the second axis separated field 29 from fields 30 and 21. Although, the principal components analysis suggested that the fields 30 and 29 were the

most dissimilar, fields 21 and 30 were selected as contrasting soil types because of the more significant differences in pH, extractable P, Mg and nitrate.

Field	Stage	Crop	pH	P	K	Mg	NH_4	NO_3
				mg ℓ^{-1}	mg ℓ^{-1}	mg ℓ^{-1}	mg kg^{-1}	mg kg^{-1}
29	1	Grass	6.8	9.8	169.0	255	5.38	8.99
21	5	Potatoes	6.3	8.5	68.8	179	1.97	9.89
30	6	Undersown oats	7.0	18.7	93.1	323	2.89	13.87
		F (2, 6 d.f.)	23.7	8.8	15.5	30	1.86	9.62
		P	***	*	**	***	-	*

Table 10.7: Summary of soil analysis for pH and extractable mineral contents, August 1997. n = 3: *** P < 0.001, ** P < 0.010 and * P < 0.050.

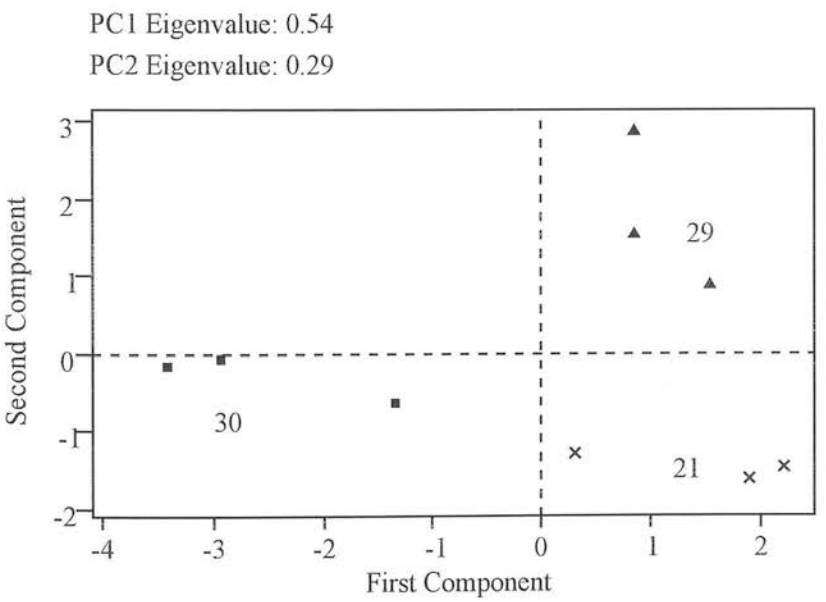


Figure 10.2: Principal components analysis of soils from three fields. Variates and field codes as Table 10.6 and Table 10.7.

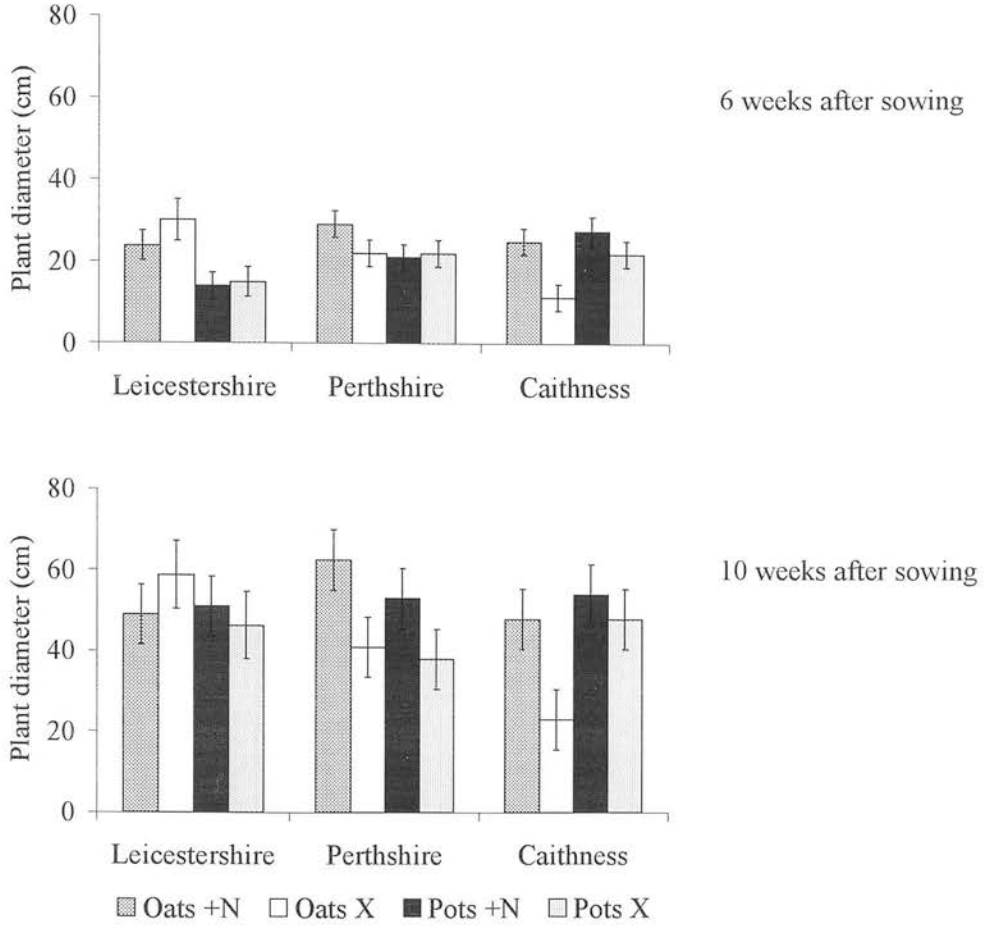
10.4.2.2. Plant growth and seed set in selected soils

Seedling establishment varied significantly between populations, but not between soils or soil nitrogen treatments. Seedling establishment was highest for the Perthshire and lowest for the Leicestershire populations.

Six weeks after sowing, plant diameter varied significantly between populations according to soil and according to soil nitrogen treatment. Ten weeks after sowing plant diameter varied significantly according to soil nitrogen treatment only. Differences between populations in plant diameter according to treatments are shown in Figure 10.3. At six weeks, the Leicestershire population was significantly larger in soil that had previously grown oats and the Caithness population was significantly larger in soils that had had additional nitrogen applied. At ten weeks, plants were significantly larger where additional nitrogen had been supplied. Similarly the number of branches per plant was significantly increased by application of additional nitrogen (results not presented).

The number of buds and flowers per plant were recorded at six and ten weeks after sowing. At six weeks, flowering was largely restricted to the Leicestershire population. At ten weeks the number of buds and flowers per plant differed significantly between populations and between soil nitrogen treatments (Figure 10.4). The number of buds and flowers per plant was highest for the Leicestershire population. The number of buds and flowers produced by the Perthshire population at the same time was minimal. Significantly more buds and flowers were also produced for plants treated with additional nitrogen.

The number of seeds per capsule differed significantly between populations, between soils and between soil nitrogen treatments in August 1998, but in October 1998 (at final harvest) less seeds were recorded per capsule and significant differences were recorded only between populations. In August and October 1998, the Leicestershire population produced the highest number of seeds per capsule and the Perthshire population produced capsules with the least. In August 1998 there were complex interactions between populations and treatments. For the Leicestershire and Caithness populations and for plants grown in the oat soil, significantly more seeds per capsule were recorded with additional nitrogen. For the Perthshire population significantly more seeds per capsule were recorded for plants grown in the potato soil (Figure 10.5).

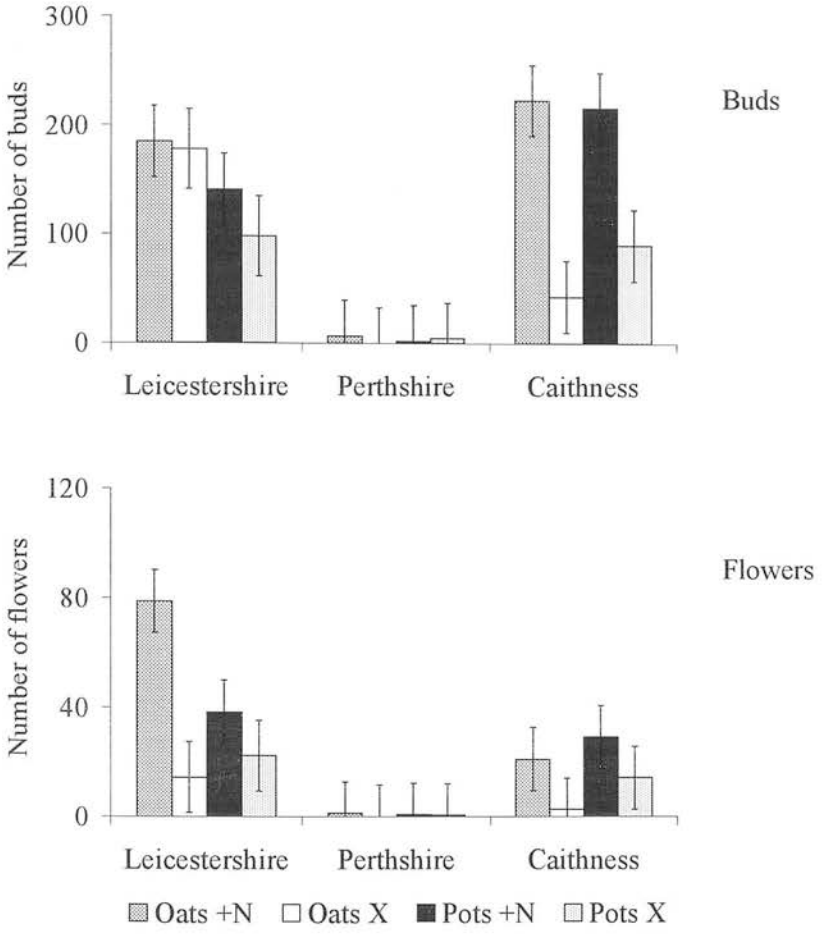


F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	6 weeks		10 weeks	
Population	2	0.82	-	1.20	-
Soil	1	3.10	-	0.10	-
Additional nitrogen	1	2.58	-	5.84	*
Population x soil	2	8.67	***	2.71	-
Population x additional nitrogen	2	4.18	*	2.28	-
Soil x additional nitrogen	1	0.96	-	0.18	-
Population x soil x additional nitrogen	2	1.42	-	1.29	-
Residual	42				

(6 missing values)

Figure 10.3: Differences in plant diameter for populations of *Stellaria media* at six and ten weeks after sowing, according to soil and soil nitrogen treatments.
Mean plant diameter ± S.E.: n = 5.
Oats (soil from Field 30), Pots (soil from Field 21).
+N: additional nitrogen, X: no additional nitrogen.

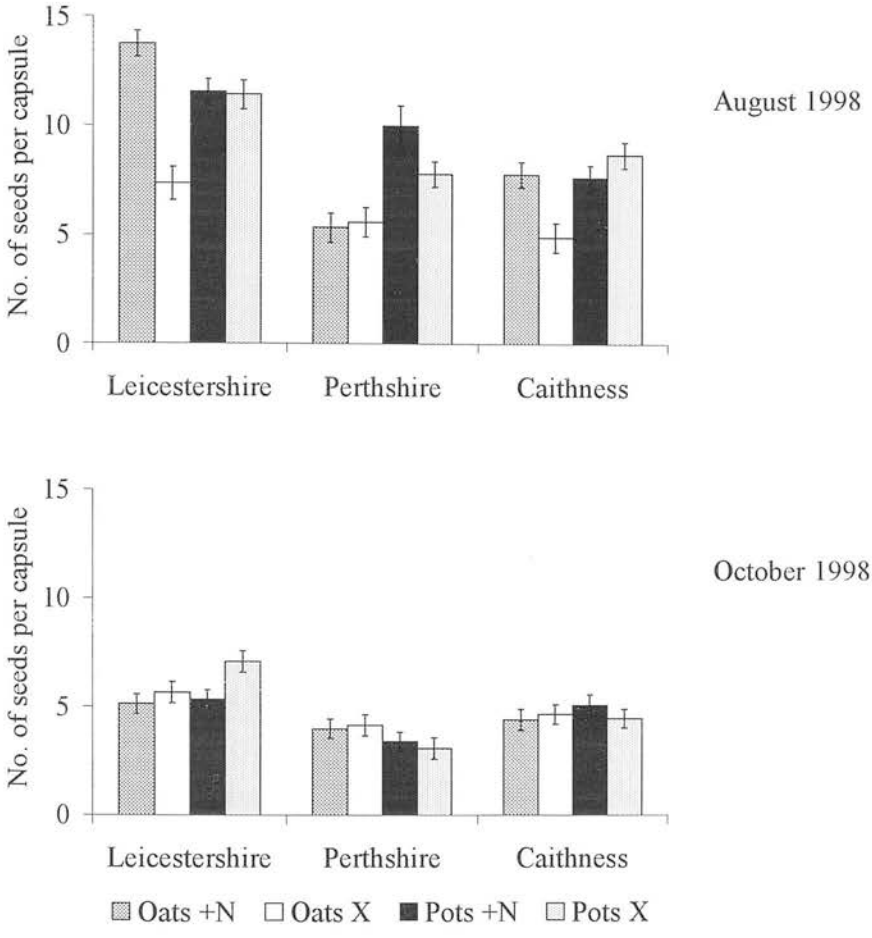


F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	Buds		Flowers	
Population	2	25.48	***	16.08	***
Soil	1	0.54	-	3.50	-
Additional nitrogen	1	10.07	**	4.51	*
Population x soil	2	1.70	-	4.25	*
Population x additional nitrogen	2	6.20	**	5.36	**
Soil x additional nitrogen	1	0.06	-	15.56	***
Population x soil x additional nitrogen	2	0.47	-	14.11	***
Residual	42				

(6 missing values)

Figure 10.4: Differences in number of buds and flowers per plant for populations of *Stellaria media* at ten weeks after sowing, according to soil and soil nitrogen treatments. Mean number of buds or flowers per plant ± S.E.: n = 5. Other details as Figure 10.3.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	August		October	
Population	2	53.31	***	23.87	***
Soil	1	36.08	***	0.07	-
Additional nitrogen	1	24.60	***	1.35	-
Population x soil	2	4.46	*	3.64	*
Population x additional nitrogen	2	5.03	**	2.81	-
Soil x additional nitrogen	1	14.20	***	0.01	-
Population x soil x additional nitrogen	2	14.26	***	1.59	-
Residual	141				

(27 missing values)

Figure 10.5: Differences in the number of seeds per capsule for populations of *Stellaria media*, according to soil and soil nitrogen treatments.
Mean number of seeds per capsule ± S.E.: n = 15.
Other details as Figure 10.3.

Figure 10.6 shows the differences between populations and between treatments for the total weight of seed produced by individual plants. The weight of seed produced by the Caithness and Leicestershire populations was significantly greater than that produced by the Perthshire

population. There were no significant differences in the total weight of seed produced according to the soil in which the plants were grown, but plants responded to the application of additional nitrogen by producing a significantly greater weight of seeds. The relative increase in the total weight of seeds produced was significantly less for Perthshire population.

Figure 10.7 shows the differences between populations and between treatments for the dry weight of above ground vegetative material following final harvest. The dry weight of above ground vegetative material after final seed harvest was significantly higher for the Perthshire population. Above ground vegetative dry weight was also significantly higher following application of additional nitrogen. There was a significant interaction between population and application of additional nitrogen, with a relatively greater increase in above ground vegetative dry weight for the Perthshire population.

Figure 10.8 shows the relationship between above ground vegetative dry weight and total seed weight. Linear relationships were fitted for each population. The Caithness population had the steepest gradient and the Perthshire population the least.

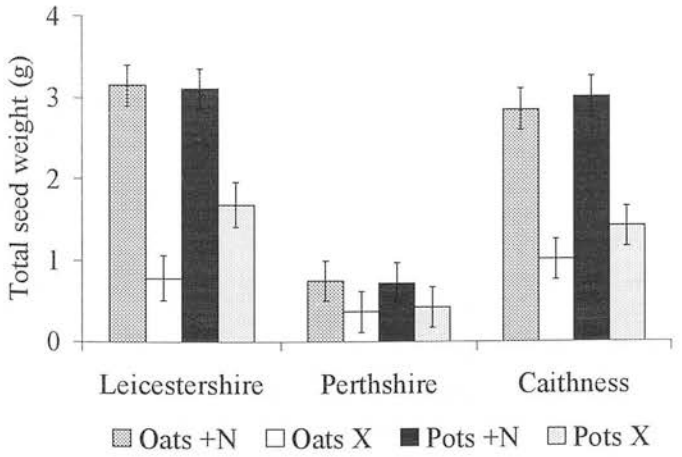


Figure 10.6: Total weight of seed produced by individual plants from three populations of *Stellaria media*, according to soil and soil nitrogen treatments.
Mean total weight of seed produced ± S.E.: n = 5.
Other details as Figure 10.3.

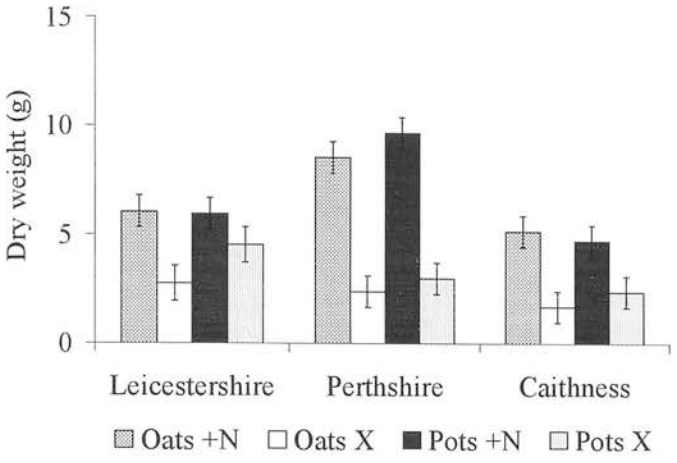


Figure 10.7: Total dry weight of above ground vegetative material after final seed harvest for three populations of *Stellaria media*, according to soil and soil nitrogen treatments.
Mean dry weight of above ground vegetative material ± S.E.: n = 5.
Other details as Figure 10.3.

Factor	d.f.	Seed weight		Vegetative dry weight	
Population	2	53.24	***	11.02	***
Soil	1	2.86	-	2.11	-
Additional nitrogen	1	84.72	***	86.14	***
Population x soil	2	0.73	-	0.33	-
Population x additional nitrogen	2	11.74	***	9.10	***
Soil x additional nitrogen	1	2.23	-	0.97	-
Population x soil x additional nitrogen	2	0.86	-	0.73	-
Residual	42				

(6 missing values)

Table 10.8: Summary of the analysis of variance for differences between populations of *Stellaria media* for total weight of seed produced and above ground vegetative dry weight at final harvest, according to soil in which plants were grown, with or without additional nitrogen.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$.

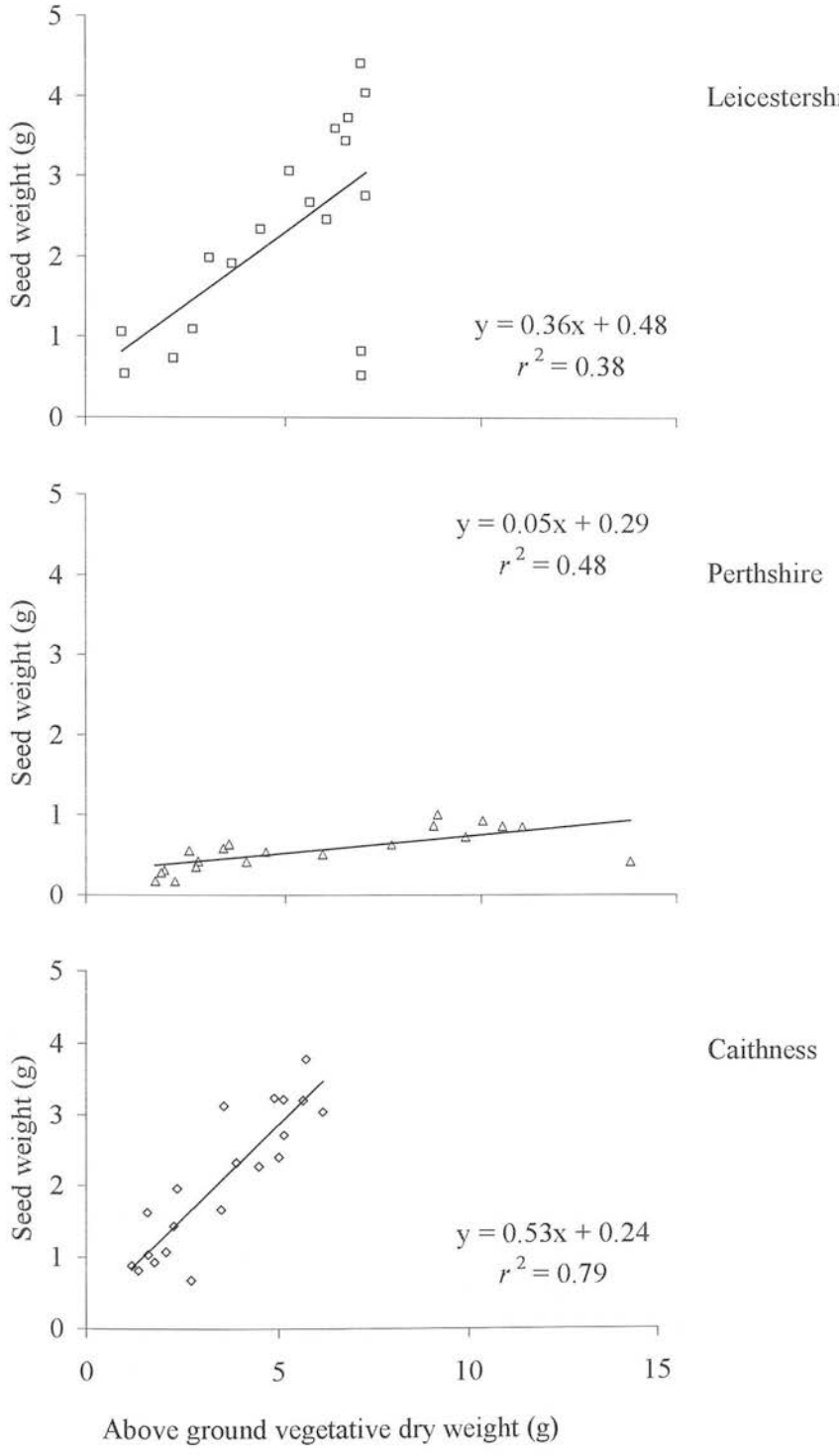


Figure 10.8: Relationship between above ground vegetative dry weight and total seed weight for *Stellaria media* populations.

10.4.2.3. Seed size and weight

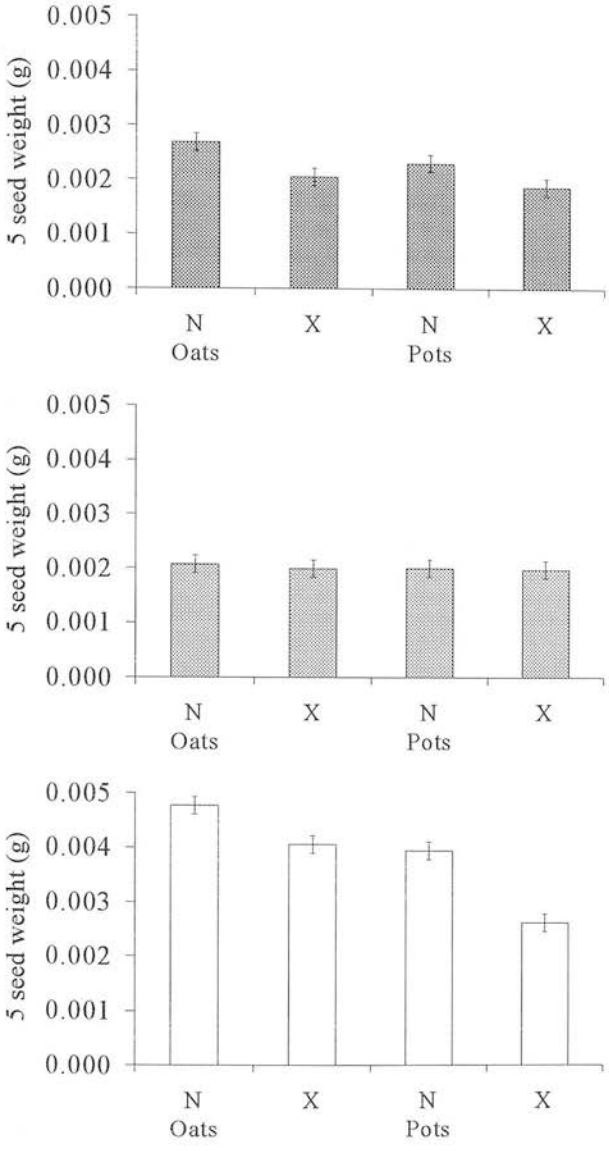
Table 10.9 shows mean seed diameters for each population according to soil and soil nitrogen treatments. Seed diameters varied significantly between populations and between treatments, although the effect of previous soil and soil nitrogen treatment varied according to population. Seed diameter was significantly wider for the Caithness population. For the Caithness and Leicestershire populations, diameters of seed produced in the oat soil were significantly wider than for seed produced in the soil that had previously grown potatoes. For these same populations and for seed that was produced in the potato soil, seed diameters were also significantly wider following additional nitrogen applications. Significant effects of additional nitrogen application in the oat soil were restricted to the Caithness population.

Population	Oats		Pots		Mean
	+N	X	+N	X	
Leicestershire	0.99	0.98	0.94	0.86	0.94
Perthshire	0.94	0.87	0.88	0.95	0.91
Caithness	1.29	1.19	1.18	0.95	1.15
Mean	1.07	1.01	0.99	0.92	0.99

F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	65.65	***
Soil	1	19.24	***
Additional nitrogen	1	14.82	***
Population x soil	2	8.48	***
Population x additional nitrogen	2	7.17	***
Soil x additional nitrogen	1	0.20	-
Population x soil x additional nitrogen	2	4.81	**
Residual	108		

Table 10.9: Mean seed diameters for seed produced in selected soils, with or without additional nitrogen for three populations of *Stellaria media*
Oats (soil from Field 30), Pots (soil from Field 21).
+N: additional nitrogen, X: no additional nitrogen.
n = 10.



F ratios with significance indicated as *** P < 0.001; ** P < 0.010 and * P < 0.050

Factor	d.f.	*F*	P
Population	2	148.57	***
Soil	1	25.97	***
Additional nitrogen	1	31.75	***
Population x soil	2	12.23	***
Population x additional nitrogen	2	8.72	***
Soil x additional nitrogen	1	0.36	-
Population x soil x additional nitrogen	2	1.69	-
Residual	24		

Figure 10.9: Five seed weights for seed produced in selected soils, with or without additional nitrogen for three populations of *Stellaria media.*
Mean five seed weight ± S.E. n = 3. Other details as Table 10.9

Figure 10.9 shows mean five seed weights for each population according to soil and soil nitrogen treatments. Five seed weights also varied significantly between populations and between treatments, though again the effect of previous soil and soil nitrogen treatment varied according to population. Five seed weight was highest for the Caithness and lowest for the Perthshire population, with individual mean seed weights of 0.77 mg for the Caithness population, 0.40 mg for the Perthshire population and 0.45 mg for the Leicestershire population. No significant treatment effects were recorded for the Perthshire population. For the Caithness population significant differences were recorded between soils, with heavier seeds produced in the soil that had previously grown oats. For the Caithness and Leicestershire populations, heavier seeds were also produced following applications of additional nitrogen and this effect was significant in both soils.

10.4.2.4. Seed germination

Table 10.10, Table 10.11 and Table 10.12 show the parameters for Gompertz curves fitted to the cumulated germination time course for *S. media* seeds produced by plants grown in the two contrasting soils, with or without additional application of mineral nitrogen. The seeds were tested at four constant temperatures, in distilled water or in 10 mmol l^{-1} KNO_3 solution and results are presented according to population. Table 10.13 summarises the results from analysis of variance for the differences between populations for fitted Gompertz parameters according to the soil in which seeds were produced, with or without application of additional nitrogen and according to test temperature and germination in either distilled water or 10 mmol l^{-1} KNO_3. Table 10.13 shows that there were significant differences between populations for germination synchronicity and final percentage germination. Germination synchronicity, germination speed and final germination percentage significantly differed according to temperature and whether seeds were germinated in distilled water or potassium nitrate solution

Consistent with previous Chapters, germination of the Caithness population remained generally less synchronous than that of the other two populations, except at 30 °C where germination of seeds from the Caithness population was relatively synchronous. Overall synchronicity of seed germination tended to be promoted by nitrate ions and greatest at 10 and significantly reduced at 30 °C. The speed of germination was greatest at 20 and least at 5 °C. Final germination percentages were highest for the Perthshire population and least for

the Leicestershire population, with overall germination percentages maximised at 5 and 10 °C and significantly reduced at 30 °C. The presence of nitrate ions in the germination test significantly increased the final germination percentage.

Significant first order effects related to the maternal environment were restricted to a marginally significant effect of soil on final germination percentage. Overall there was a trend to higher final percentage germination for seeds produced from plants grown in the oat soil. It was however evident that this trend interacted significantly with population, temperature and whether seeds were germinated in distilled water or potassium nitrate solution. This is also shown in Figure 10.10

Second order interactions showed that the Caithness population tended to germinate with greater synchronicity in the presence of nitrate ions and that the relatively synchronous and high percentage germination at 30 °C was also associated with relatively faster speed of germination, especially when compared with the Perthshire population. In contrast, at 5 °C the Caithness population germinated relatively slowly compared with the other populations. Germination speed was relatively slow for seeds of the Leicestershire population produced from plants grown in the oat soil and also for seeds produced from soils given an additional application of mineral nitrogen. Germination speed for all seeds from the Leicestershire population, regardless of soil type or soil nitrogen treatment, tended to be relatively slow when tested in potassium nitrate solution. The reverse was the case for the Caithness population, where nitrate ions tended to promote the speed of germination. For the Caithness population, final percentage germination was significantly higher for seeds produced from plants grown in the oat soil and for seed produced following application of additional mineral nitrogen. In contrast, final percentage germination for the Leicestershire population was significantly higher for seeds produced without application of additional nitrogen.

(a) Distilled water

Temperature °C	Soil	Additional nitrogen	β hr^{-1}	μ hr	γ	α	$t50$ hr
5	Oats	+	0.0585	164.82	49.81	0.14	165.18
		-	0.0583	153.16	75.73	-0.03	153.52
	Pots	+	0.0696	146.96	75.02	-0.12	147.33
		-	0.0339	157.72	55.59	-0.10	158.09
10	Oats	+	0.1089	84.20	72.17	0.03	84.38
		-	0.0788	87.40	80.04	-0.14	87.59
	Pots	+	0.0833	85.95	79.73	0.22	86.13
		-	0.1320	85.77	73.73	0.00	85.95
20	Oats	+	0.0825	30.01	56.30	-3.10	30.16
		-	0.0817	31.92	80.09	-3.65	32.06
	Pots	+	0.0818	28.96	69.75	-4.65	29.12
		-	0.0808	30.59	57.35	-2.79	30.74
30	Oats	+	0.0555	123.52	6.21	-0.16	123.62
		-	0.0703	48.95	30.62	-0.77	49.05
	Pots	+	0.0320	82.55	9.90	-0.22	82.65
		-	0.0668	53.53	51.07	-2.51	53.63

(b) 10 mmol l^{-1} KNO_3

Temperature °C	Soil	Additional nitrogen	β hr^{-1}	μ hr	γ	α	$t50$ hr
5	Oats	+	0.0779	165.51	96.57	0.04	165.87
		-	0.0614	171.57	98.06	0.01	171.94
	Pots	+	0.0720	165.18	100.85	-0.07	165.54
		-	0.0786	156.25	100.99	0.35	156.61
10	Oats	+	0.1411	83.24	99.96	-0.08	83.42
		-	0.1107	88.66	100.12	-0.02	88.85
	Pots	+	0.1514	84.38	99.92	-0.22	84.56
		-	0.1301	85.16	98.71	-0.05	85.35
20	Oats	+	0.0833	34.63	80.63	-2.45	34.76
		-	0.0825	29.98	92.07	-4.57	30.12
	Pots	+	0.0843	31.16	85.73	-3.88	31.30
		-	0.0817	31.98	92.98	-4.20	32.12
30	Oats	+	0.0076	192.75	49.74	-2.69	192.86
		-	0.0547	56.87	52.93	-0.90	56.97
	Pots	+	0.0392	90.34	59.28	-0.36	90.43
		-	0.0395	69.09	69.61	-2.22	69.20

Table 10.10: Fitted Gompertz parameters for germination of *Stellaria media* seed from the Leicestershire population produced in different soils, with or without application of additional mineral nitrogen and according to temperature in distilled water or 10 mmol l^{-1} KNO_3 solution.
n = 3: Oats (soil from Field 30), Pots (soil from Field 21).

(a) Distilled water

Temperature °C	Soil	Additional nitrogen	β hr^{-1}	μ hr	γ	α	$t50$ hr
5	Oats	+	0.0745	158.10	98.01	-0.29	158.47
		-	0.0758	168.23	101.73	-0.25	168.60
	Pots	+	0.0669	158.30	96.36	0.51	158.66
		-	0.0780	161.09	97.63	0.08	161.45
10	Oats	+	0.1379	81.82	97.27	0.17	82.00
		-	0.1482	85.02	100.06	0.00	85.20
	Pots	+	0.1164	92.31	98.56	0.07	92.49
		-	0.1162	82.89	100.07	-0.15	83.07
20	Oats	+	0.0824	28.39	88.64	-4.36	28.54
		-	0.0830	30.75	94.48	-4.52	30.89
	Pots	+	0.0853	34.04	80.64	-2.70	34.17
		-	0.0820	32.33	101.50	-2.96	32.47
30	Oats	+	0.0244	88.64	50.38	-0.99	88.74
		-	0.0285	100.85	38.71	-0.42	100.94
	Pots	+	0.0252	131.13	22.68	-0.05	131.22
		-	0.0195	146.11	43.90	-0.64	146.20

(b) 10 mmol l^{-1} KNO_3

Temperature °C	Soil	Additional nitrogen	β hr^{-1}	μ hr	γ	α	$t50$ hr
5	Oats	+	0.0807	160.03	99.65	0.20	160.39
		-	0.0773	165.70	102.00	-0.17	166.07
	Pots	+	0.0640	172.83	97.09	0.04	173.19
		-	0.0785	163.45	99.09	-0.15	163.82
10	Oats	+	0.1489	84.08	97.53	0.10	84.26
		-	0.1522	88.37	100.09	0.00	88.56
	Pots	+	0.1010	89.10	99.85	-0.06	89.28
		-	0.1520	84.14	100.71	-0.26	84.33
20	Oats	+	0.0843	31.16	105.53	-5.12	31.31
		-	0.0843	32.51	101.14	-3.93	32.65
	Pots	+	0.0850	33.55	101.16	-3.18	33.69
		-	0.0834	31.38	105.03	-4.32	31.52
30	Oats	+	0.0332	107.22	77.42	-0.17	107.31
		-	0.0273	98.48	71.41	-0.91	98.58
	Pots	+	0.0175	93.72	62.75	-9.00	93.86
		-	0.0190	103.71	78.93	-5.37	103.82

Table 10.11: Fitted Gompertz parameters for germination of *Stellaria media* seed from the Perthshire population produced in different soils, with or without application of additional mineral nitrogen and according to temperature in distilled water or 10 mmol l^{-1} KNO_3 solution.
n = 3: Other details as for Table 10.10.

(a) Distilled water

Temperature °C	Soil	Additional nitrogen	β hr^{-1}	μ hr	γ	α	$t50$ hr
5	Oats	+	0.0445	218.77	41.21	0.32	219.13
		-	0.0367	194.17	63.41	0.02	194.54
	Pots	+	0.0239	170.29	58.22	-0.28	170.66
		-	0.0423	203.46	53.58	0.02	203.83
10	Oats	+	0.0611	99.55	69.11	0.50	99.73
		-	0.0583	98.17	68.82	-0.13	98.35
	Pots	+	0.0652	95.05	66.60	-0.12	95.23
		-	0.0551	96.86	61.68	-0.25	97.05
20	Oats	+	0.0700	33.58	36.32	-1.81	33.73
		-	0.0792	34.34	24.09	-0.70	34.48
	Pots	+	0.0788	38.54	45.68	-0.71	38.66
		-	0.0798	50.76	16.12	-0.78	50.90
30	Oats	+	0.0400	52.71	84.31	0.10	52.80
		-	0.0440	49.96	95.51	-3.16	50.06
	Pots	+	0.0467	47.56	84.93	-1.49	47.66
		-	0.0200	128.31	21.00	0.09	128.41

(b) 10 mmol l^{-1} KNO_3

Temperature °C	Soil	Additional nitrogen	β hr^{-1}	μ hr	γ	α	$t50$ hr
5	Oats	+	0.0560	183.55	97.13	0.06	183.91
		-	0.0754	162.08	101.28	-0.37	162.45
	Pots	+	0.0549	189.83	100.41	0.09	190.19
		-	0.0676	180.00	100.23	-0.08	180.37
10	Oats	+	0.1125	94.77	95.59	0.19	94.95
		-	0.1196	83.85	99.87	0.05	84.03
	Pots	+	0.0997	93.53	95.55	0.94	93.71
		-	0.1299	94.48	99.84	0.00	94.67
20	Oats	+	0.0843	32.56	98.42	-2.51	32.69
		-	0.0824	33.05	103.09	-3.93	33.20
	Pots	+	0.0830	33.80	92.91	-3.62	33.94
		-	0.0854	35.10	79.98	-2.38	35.24
30	Oats	+	0.0864	41.07	95.55	0.01	41.16
		-	0.0951	46.03	101.50	-0.75	46.12
	Pots	+	0.0926	43.35	95.88	1.34	43.43
		-	0.0761	47.74	42.39	-0.12	47.83

Table 10.12: Fitted Gompertz parameters for germination of *Stellaria media* seed from the Caithness population produced in different soils, with or without application of additional mineral nitrogen and according to temperature in distilled water or 10 mmol l^{-1} KNO_3 solution.
n = 3: Other details as for Table 10.10.

Factor	d.f.	β hr^{-1}		μ hr		$\alpha + \gamma$	
Population	2	6.04	**	1.90	-	57.81	***
Temperature	3	181.86	***	648.50	***	139.62	***
Soil	1	2.21	-	0.23	-	4.54	*
Additional nitrogen	1	1.86	-	2.50	-	0.60	
Test nitrogen	1	48.38	***	1.14	-	428.54	***
Population x temperature	6	23.02	***	18.83	***	43.97	***
Population x soil	2	2.52	-	5.30	**	9.40	***
Population x additional nitrogen	2	0.39	-	7.13	***	12.82	***
Population x test nitrogen	2	20.35	***	7.81	***	40.26	***
Temperature x soil	3	0.47	-	0.29	-	2.73	*
Temperature x additional nitrogen	3	0.21	-	1.13	-	0.48	-
Temperature x test nitrogen	3	8.22	***	0.20	-	5.03	**
Soil x additional nitrogen	1	0.67	-	8.38	**	11.16	***
Soil x test nitrogen	1	0.04	-	1.76	-	0	-
Additional nitrogen x test nitrogen	1	0.20	-	3.28	-	0.44	-
Population x temperature x soil	6	1.64	-	2.98	**	6.31	***
Population x temperature x additional nitrogen	6	2.96	**	8.62	***	6.58	***
Population x temperature x test nitrogen	6	4.76	***	2.09	-	15.36	***
Population x soil x additional nitrogen	2	0.63	-	3.21	*	15.01	***
Population x soil x test nitrogen	2	0.44	-	0.49	-	0.09	-
Population x additional nitrogen x test nitrogen	2	1.55	-	0.25	-	1.53	-
Temperature x soil x additional nitrogen	3	3.46	*	5.35	***	0.52	-
Temperature x soil x test nitrogen	3	0.07	-	4.04	**	0.30	-
Temperature x additional nitrogen x test nitrogen	3	0.17	-	1.12	-	0.56	-
Soil x additional nitrogen x test nitrogen	1	0.01	-	0.15	-	2.18	-
Residual	194						

(25 missing values)

Table 10.13: Summary of the analysis of variance for the differences between populations of *Stellaria media* for fitted Gompertz parameters according to the soil in which seeds were produced, with or without application of additional nitrogen and according to test temperature and germination in either distilled water or 10 mmol l^{-1} KNO_3.
F ratios with significance as *** $P < 0.001$; ** $P < 0.010$ and * $P < 0.050$
Analysis was restricted to order 3 interactions.

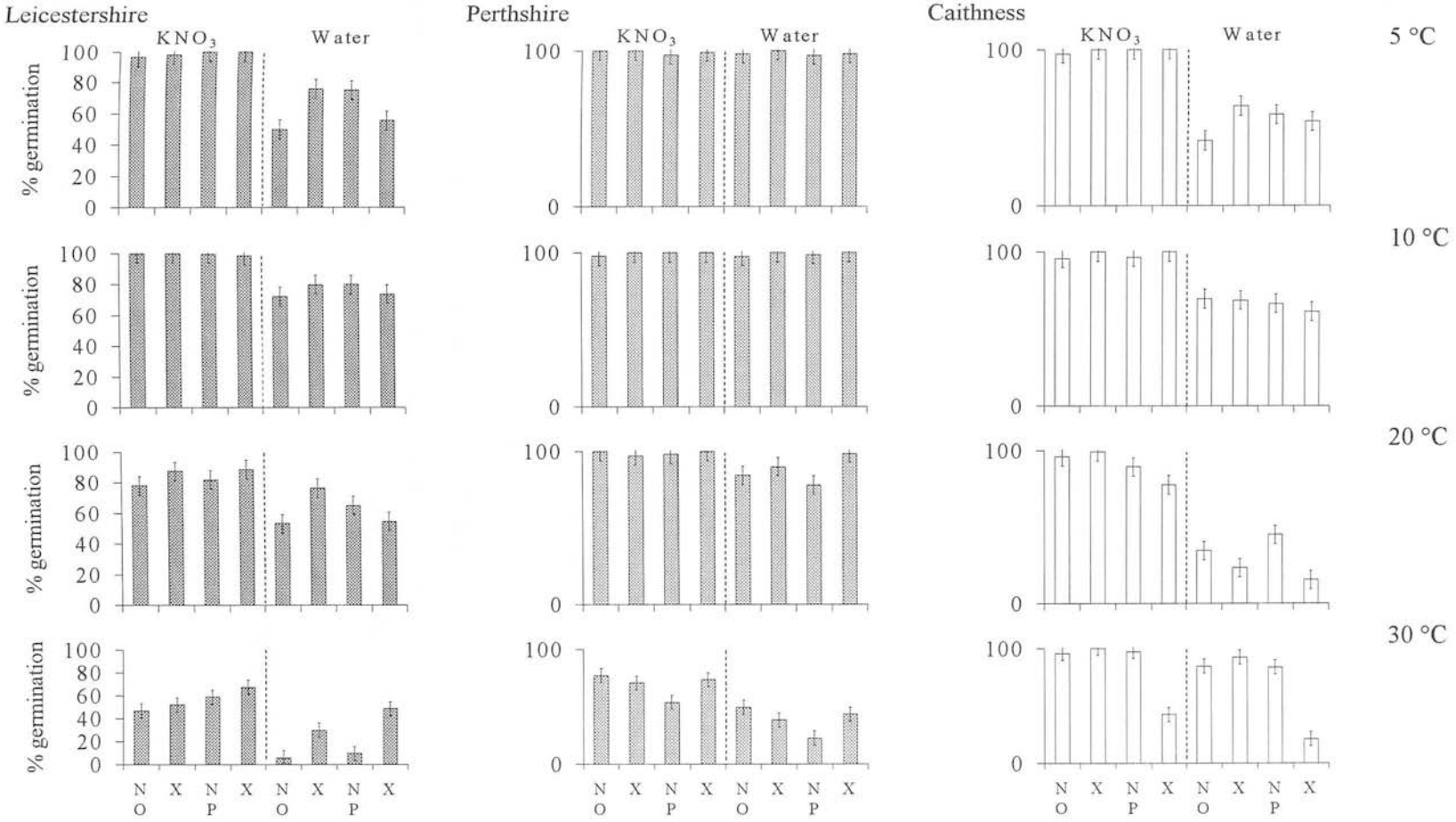


Figure 10.10: Final % germination estimated from Gompertz parameters ($\alpha + \gamma$) for *Stellaria media* seeds produced from plants grown in different soils, with or without application of additional nitrogen and tested at four constant temperatures, in distilled water or 10 mmol l^{-1} KNO_3
Mean ($\alpha + \gamma$) ± S.E.: n = 3: N = with additional nitrogen at 100 kg ha^{-1} (34.5 % NH_4NO_3), X = without: O = after oats, P = after potatoes

Third order interactions reveal the complexity of the effects of soil type and soil nitrogen status on the Gompertz parameters describing seed germination. It is clear that the majority of these interactions included a temperature term and that the differential effects of soil type and whether additional nitrogen was applied were specific to the higher temperatures. For both the Caithness and Perthshire populations, germination speed at 30 °C was quicker (and for the Caithness population, final germination percentage was higher) for seeds produced from plants grown in the oat soil. In contrast, for the Leicestershire population, germination speed at 30 °C was quicker and final germination percentage was higher for seeds produced from plants grown in the soil that had previously grown potatoes. For the Caithness population it was clear that application of additional nitrogen increased the germination speed and final germination percentage of seeds produced by plants grown in the potato soil. In contrast, for the Leicestershire population, application of additional nitrogen decreased the germination speed and final germination percentage of seeds produced by plants grown in the oat soil. Similarly, application of additional mineral nitrogen reduced the final germination percentage for seeds from the Perthshire population produced by plants grown in the potato soil.

10.5. Discussion

The results from this experiment again show the extent of variation between populations of *S. media*, both in patterns of seed germination, seed production and plant growth.

It is interesting to note that seed germination in both laboratory tests showed that nitrogen increased final germination percentage, but measurements in the pot experiment showed no relationship between the number of seedlings that emerged and the different soil and soil nitrogen treatments. This corresponds to observations from several other authors, that under field conditions, factors other than nitrate effectively limit seed germination (Fawcett & Slife, 1978b; Jørnsgård, Rasmussen, Hill & Christiansen, 1996).

10.5.1. Effect of nitrogen and nitrogen source on seed germination

In the laboratory experiment to quantify the effect of nitrogen and nitrogen source on seed germination, nitrogen was shown to increase the speed of germination and final germination percentage. This supports previous observations by Roberts & Lockett (1975) who showed that germination of dry stored *S. media* seeds was higher in 20 mmol l^{-1} potassium nitrate solution than germination at the same temperatures in distilled water. The results also

correspond with previously published results for a wide range of weed species, including *Sinapis arvensis* (Goudey *et al.*, 1988), *Sisymbrium officinale* (Karssen & de Vries, 1983), *Chenopodium album* (Williams & Harper, 1965), *Spergula arvensis* (Vincent & Roberts, 1977) and *Galium aparine* (Froud-Williams, 1985; Ferris, 1988).

It was clear that nitrogen supply as either ammonium ions or nitrate ions at 1 mmol l^{-1} promoted germination less than higher concentrations. However supply of nitrogen as 1 mmol l^{-1} KNO_3 (but not 1 mmol l^{-1} NH_4Cl) still significantly increased final germination percentages and germination speed compared with distilled water, which implies that *S. media* germination is very sensitive to nitrate concentrations. It was interesting to note that there was a poor relationship between seed germination and concentrations at rates greater than 10 mmol l^{-1}. Although there was no suggestion that seed germination was inhibited by supply of nitrate or ammonium ions at higher concentrations, it should be noted that this has been recorded in previous experiments, notably in *Sinapis arvensis* (Goudey *et al.*, 1986). It is also interesting to note that Ferris (1988) reported a poor relationship between *G. aparine* seed germination and potassium nitrate concentration.

The results from this experiment support the observation that seed germination responded preferentially to nitrogen supplied as the nitrate ion compared to the ammonium ion. However, it was notable that the ammonium ion also stimulated seed germination and previous reports of this are limited (e.g. Hendricks & Taylorson, 1974 in Karssen & de Vries, 1983; Goudey *et al.*, 1986). Schimpf & Palmblad (1980) observed no significant differences between *Amaranthus retroflexus* and *Setaria glauca* emergence in soil treated with either nitrate fertiliser or ammonium fertiliser (applied with nitrification inhibitors), though it should be noted that the overall effect of nitrogen application on seedling emergence was not significant. Karssen & de Vries (1983) reported no significant effect of ammonium sulphate supplied at either 2.5 or 5 mmol l^{-1} on germination of *S. officinale*. It is possible that this difference may relate to the difference in associate ions (sulphate compared to chloride), or this may reflect a difference in species biology. It was clear that nitrate generally had a greater effect on germination of *S. officinale* compared with *S. media*, especially in combination with light and temperature. *S. officinale* is an obligate summer annual with high levels of dormancy in the autumn. It is speculated that *S. officinale* has evolved a specific need for nitrate that is associated with nitrate release in the spring. It is possible that *S. media* has evolved to be less sensitive to nitrogen source and can substitute nitrate requirements with ammonium. The molecular role of nitrogen in germination is still

poorly understood and as such it is not clear whether substituting nitrate with ammonium requires the presence of different metabolic pathways.

10.5.2. Effect of soil nitrogen on plant growth, seed production, seed size and seed germination

Plant size and seed production were positively related, though there was substantial variation from a significant linear relationship, such as those defined for other weed species by Watkinson & White (1985, in Cousens & Mortimer, 1995). Plant size and seed production both increased with applications of additional nitrogen, but no significant differences were recorded according to soil type. This suggests that differences in soil mineral concentrations within an organic rotation were minimal compared to differences between organic and conventional farming systems. However it is important to note that these experiments were conducted in the absence of crop plants. Field experiments have shown that crop plant growth was relatively more responsive to nitrogen and as such, that weed problems have tended to be more severe in low nitrogen treatments with less crop competition (Jørnsgård *et al.*, 1996). Grundy, Froud-Williams & Boatman (1995) also showed that whilst application of higher rates of nitrogen (160 compared with 40 kg N ha^{-1}) increased plant dry weight for *Viola arvensis* grown in a winter wheat crop, seed production was not significantly affected.

Differences between populations for plant size and seed production were largely consistent with previous results (Chapter 8), with the Perthshire population producing less seed despite greater above ground vegetative dry matter. Differences in plant phenology and reproductive effort have previously been used to explain these differences and this was consistent with observations during this experiment. It was also clear from observations that applications of nitrogen tended to promote earlier or more vigorous flowering, at least in the Leicestershire and Caithness populations.

Differences between populations for the number of seeds per capsule were consistent across the season, but reductions in the number of seeds per capsule in October compared to August may again reflect difficulties in seed collection, with greater seed dispersing and escaping collection in early autumn. However, given that efforts were made to minimise seed loss, this may have been a seasonal effect, possibly occurring as a consequence of increasing resource limitation. Despite the lack of consistency over the season and complex interactions between populations and treatments, in August significantly more seeds per

capsule were recorded with additional nitrogen for plants grown in the oat soil from the Leicestershire and Caithness populations and this may support the resource supply argument.

Significant differences were recorded between populations and treatments for seed size and weight. The general pattern of larger and heavier seed produced by the Caithness population was consistent with previous results (Chapters 2 & 8). Populations interacted with treatments and no treatment effects were recorded for the Perthshire population. Treatment effects on seed size and weight were most apparent for the Caithness population, though it may be argued that this was an artefact of greater measurement accuracy for larger seeds. Nevertheless it was apparent that for the Caithness and Leicestershire populations, larger seeds tended to be harvested from plants grown in the more nutrient rich oat soil and following applications of additional nitrogen. Grundy *et al.* (1995) also observed that heavier seeds of *V. arvensis* tended to be produced following field applications of high rates of nitrogen.

Interestingly for these same populations, where increased seed size was associated with increased soil nutrient concentration, seed size was reduced following low dose herbicide applications (Chapter 8). Given that in both cases, seeds were collected from a common environment and within the same limited time interval, this might suggest that seed size is a more plastic characteristic in these two populations. Interestingly Chapter 8 implicated changes in seed size with subsequent changes in germination, as smaller seeds tended to be less germinable and Stanton (1984), Champion, Froud-Williams & Holland (1997) and Andersson (1996) have also recorded similar seed size effects on germination. Consequently changes in seed size according to the nutrient status of the soil in which the parent plant was grown may have significant effects on seed germination. There may also be other seed size related consequences for patterns of seed dispersal (Thompson, Band & Hodgson, 1993) and seed predation (Reader, 1993).

Regarding subsequent patterns of seed germination, it was clear that there was a general lack of treatment effects for seeds produced in the different soils, with or without application of additional nitrogen. Certainly the limited effects observed were not evident when seeds were germinated in 10 mmol l^{-1} KNO_3 and when germinated in distilled water they were only evident at either extreme temperatures or contradictory results were recorded for the different populations. This again demonstrated the importance of conducting germination tests in a range of conditions.

The marginally significant trend to increased final germination percentage for seeds grown in the more nutrient rich soil (oats) may support findings by Fawcett & Slife (1978b) and Saini *et al.* (1985a, b) who showed that high levels of soil nitrates promoted higher levels of germination in subsequent seed generations for several weed species. Interestingly this minor effect was clearest for the Caithness population and this was the same population where higher soil nutrients were associated with increased seed size. However for the Leicestershire population where similar seed size effects were related to soil nutrient status, final percentage germination and germination speed were reduced for the larger seeds produced following applications of additional nitrogen. As such there was limited support for generalisations about seed germination and relative seed size as reported by Stanton (1984).

It is suggested that further experiments are required with greater control of soil nutrients (e.g. using sand and nutrient solution) to establish whether these results are consistent for *S. media*. However the practical significance of these minor differences, given that they were not detectable at normal temperatures in nitrate solution (that approximated to soil solution) is questionable. That said, it could be argued that such minor differences could accumulate over time if higher levels of germination are selectively advantageous in more nutrient rich situations.

Differences between populations for seed germination were largely consistent with previous results (Chapters 2, 5, 6 & 8), with the Perthshire population tending to germinate to a high level in all test conditions and unusually high levels of germination recorded for the Caithness population at 30 °C. As such these general characteristics will not be discussed again in this Chapter.

10.6. Conclusions

Patterns of plant growth, seed production and seed germination varied between populations and in response to nitrogen.

Plant growth and seed production were compared in soils taken from two contrasting stages of an organic rotation, with and without conventional applications of nitrogen fertiliser. Plant growth and seed production were significantly increased by applications of additional nitrogen, but no differences were recorded between soils. This suggested that the consequences of different soil mineral concentrations within the organic rotation were minimal compared to the differences between organic and conventional farming systems in terms of weed growth and seed production.

Significant differences were recorded between populations and soil treatments for seed size and weight. For the late flowering Perthshire population, with low reproductive effort, there were no significant treatment effects on seed size, but for the Caithness and Leicestershire populations, larger seeds tended to be harvested from plants grown in the more nutrient rich soil and following applications of additional nitrogen.

Changes in seed size were not associated with subsequent patterns of seed germination and it was clear that there was a general lack of treatment effects on seed germination for seeds produced in the different soils, with or without application of additional nitrogen.

In laboratory tests, seed germination responded preferentially to nitrogen supplied as the nitrate ion compared to the ammonium ion, although the ammonium ion promoted seed germination. Germination increased as nitrogen concentrations increased from 1 to 10 mmol l^{-1}, but there was a poor relationship between seed germination and nitrogen concentrations greater than 10 mmol l^{-1}.

In comparison with these laboratory tests, the soil experiment showed no relationship between the number of seedlings that emerged and the different soil and soil nitrogen treatments. This suggests that other factors may limit seed germination in the field.

Chapter 11. Modelling seed dormancy and germination for *Stellaria media* and *Galium aparine*.

11.1. Summary

For *Stellaria media* and *Galium aparine*, the timing and extent of germination in the soil seedbank was modelled in terms of the factors influencing dormancy (soil temperature) and germination (temperature and water potential). The dormancy model was developed from similar ideas proposed for summer annual species by Vleeshouwers (1997), whilst the germination model was developed independently following examination and assessment of existing hydrothermal time models (Gummerson, 1986). Both models were developed in a stochastic framework, which addressed statistical problems associated with least squares regression (Hunter, Glasbey & Naylor, 1984).

The seed dormancy model represented seasonal changes in the proportion of seeds that were 'non-dormant', according to soil temperature. This model then identified the proportion of these 'non-dormant' seeds that were germinable in a given set of temperature and water potential conditions. This represented the proportion of seeds germinating under test conditions, following exhumation at intervals during the year.

The timing of germination model represented the timing and extent of germination according to temperature and water potential. Germination extent was modelled from input of the proportion of seeds that were 'non-dormant' and identification of the proportion of these seeds that were germinable for a given set of temperature and water potential conditions. This proportion of germinable seeds was fixed at initiation of germination. For these germinable seeds, the timing of germination was modelled as a function of test temperature and hydrothermal time.

The dormancy model for *G. aparine* was based on previously published data (van der Weide, 1993) and includes no information on the extent of variation between populations. Model fitting for *S. media* used data from Chapter 5 and different parameter values were required to represent the behaviour of the different populations. For both species, dormancy was induced by late spring/early summer temperatures, and released in late summer. Complete

dormancy was induced in mid-summer for *S. media*, whilst for *G. aparine* germination was largely restricted to between 7 and 13 °C.

Model fitting for the timing of germination model used data from Chapter 6 for *S. media* and from Chapter 7 for *G. aparine*. This restricted model application to conditions of constant temperature and water potential. Different parameters were required to represent the behaviour of the different populations

Model implementation used a commercial modelling package (Modelmaker ver. 3.0.3) and this presents a readily accessible and adaptable framework for further model development.

11.2. Introduction

Knowledge of the timing and extent of weed seed germination is important in determining patterns of weed seedling emergence in the field (Vleeshouwers, 1997; van der Weide, 1993). Better understanding of the patterns of weed seedling emergence in the field is important to improve both the timing and choice of weed control measures. The importance of timing has been emphasised in relation to maximising the efficacy of low dose herbicide applications in conventional farming (Kudsk, 1989; Whytock & Davies, 1996) and cultivations for weed control in organic systems (Wilson, Wright & Butler, 1993). Better understanding of the population dynamics and ecology of weed species has the potential to identify novel methods of weed control, which may have environmental and economic benefits.

Following a review of data requirements for developing models of seed dormancy and germination for *Stellaria media* and *Galium aparine* (Chapter 4), a series of investigations were initiated. These data were presented in Chapters 5, 6, 7 & 10. The aim of this Chapter is to try to synthesise some of these data into models. For simplicity this will focus only on soil temperature in relation to seasonal changes in seed dormancy, and temperature and water potential in relation to the timing and extent of seed germination. However, the modelling framework adopted is sufficiently flexible to allow additional factors to be incorporated later.

Chapter 4 reviewed weed population modelling and identified that the most useful predictions of weed seedling emergence have been made using explicit ecophysiological

models of biological processes (e.g. Spitters, 1989; Benech-Arnold *et al.*, 1990; van der Weide, 1993; Bouwmeester & Karssen, 1992, 1993a, b, c, Vleeshouwers, 1997). This has tended to involve breaking weed seedling emergence into sub-models for weed seed dormancy, seed germination and pre-emergence growth. It should be noted that the focus of this study has been restricted to seed processes and therefore predictions of seedling emergence requires that the models presented in this chapter should be allied with models of pre-emergence growth. Van der Weide (1993) and Vleeshouwers (1997) have both defined appropriate models of this process that could be adapted for *S. media* and *G. aparine*.

From the models reviewed, Vleeshouwers' (1997) models of weed seed dormancy and germination in summer annual weed species were particularly influential to the approach adopted in this Chapter. However in adapting and developing these ideas for application to winter annual species, a number of key limitations were addressed.

Most fundamentally this involved estimating model parameters for more than one population in order to address the issue of intraspecific variation.

In terms of model formulation, models were developed within a stochastic framework that enabled maximum likelihood methods to be used for parameter estimation. This meant that confidence intervals could be given for parameter estimates.

For the dormancy model, model development was based on similar principles as those proposed by Vleeshouwers (1997), but efforts were made to simplify the model by reducing the number of parameters used in modelling this process.

For the germination model, the effect of water potential on the extent and timing of seed germination was incorporated. This involved adaptation and extension of the concept of hydrothermal time, as proposed by Gummerson (1986). It also incorporated dormancy effects on the rate of germination.

11.3. Model development

The model was developed in collaboration with Dr. Glenn Marion of the Department of Statistics and Modelling Science, University of Strathclyde. The models were developed from my ideas and conceptual framework, following initial work with existing models. Dr.

Marion was responsible for identifying mathematical functions for the processes involved and I assessed the suitability of these functions. Dr. Marion also implemented initial versions of the model and fitted them using purpose written FORTRAN code and routines from the NAG library (Numerical Algorithms Group, 1999). I then implemented these models in Modelmaker ver. 3.0.3. (Cherwell Scientific Publishing, 1997) with data output to Microsoft Excel 97 (Microsoft Corporation, 1997).

Seed dormancy and germination were modelled on an individual basis determined by a series of stochastic rules. The advantages of this stochastic approach were twofold, in that this represented variation between individual seeds and this formulation allowed the calculation of confidence intervals for predicted means parameter values using maximum likelihood estimation.

The model recognises three processes in predicting the extent and timing of seed germination. The first stage is concerned with identifying seasonal variation in the probability that a seed is 'non-dormant'. The second stage identifies the probability for the 'non-dormant' seed, at a given instant, that it is germinable in a given set of environmental conditions and the third stage is concerned with the timing of germination for a germinable seed. Dormancy and identification of the germinable seed fraction are considered together in section 11.3.1 as changes in seed dormancy cannot be directly observed and can only be inferred from seasonal changes in seed germination for a given set of environmental conditions.

This model involves a separation of time scales, with dormancy changing over relatively long time scales and germination occurring over much shorter time scales, such that it is assumed that any changes in dormancy during the germination process can be ignored. Germination is initiated by a stimulus, such as cultivation, and germination extent (i.e. probability that a seed is germinable) is fixed at this point. Germination timing is determined by integrating environmental effects over time. Fixing germination extent at initiation is not problematic in model application to controlled experiments with constant environmental conditions. However, the current model may need adaptation to allow field application in some circumstances.

11.3.1. Dormancy cycles and identification of the germinable seed fraction

For light requiring, summer annual seeds, Vleeshouwers (1997) related seasonal changes in seed dormancy to change in the level of a hypothetical phytochrome receptor X. Dormancy release at low temperatures was associated with synthesis (or increased availability) of the receptor protein X. Dormancy induction at high summer temperatures was associated with protein degradation (or decreased availability). As such the degree of dormancy was inversely related to the level of X, and changes in X were driven by seasonal changes in soil temperature.

A similar approach was adopted for development of this seed dormancy model, with dormancy linked to the relative abundance of two forms of a seed protein, Y. It is assumed that there is a fixed quantity of Y and the two forms are interchangeable.

$$Y_1 \underset{I}{\overset{R}{\rightleftarrows}} Y_2 \tag{11.1}$$

If the probability that a seed is dormant is assumed to equate to the relative abundance of Y_1, then I determines the rate of dormancy induction and R the rate of dormancy release. Given that there is a fixed quantity of Y, the relative abundance of Y_2 equates to the probability $q(t_d)$, that a seed is 'non-dormant' at time, t_d.

$$q(t_d) = \frac{[Y_2]}{[Y_1]+[Y_2]} \tag{11.2}$$

Given the need to separate time scales, the subscript $_d$ is used to distinguish time in the dormancy model from that in the germination model.

Given an initial value q_0, subsequent changes in $q(t_d)$ are described by:

$$\frac{dq(t_d)}{dt_d} = (1 - q(t_d))R - q(t_d)I \tag{11.3}$$

Vleeshouwers (1997) and Bouwmeester & Karssen (1992, 1993a, b, c), described rates of dormancy induction and release that were dependent on soil temperature (T_s). Similarly in

this model, rates of dormancy induction and release were related to soil temperature, albeit with different functions involving fewer parameters:

$$I = K_I \, s\,(T_s) \tag{11.4a}$$

$$R = K_R \, (1 - s\,(T_s)) \tag{11.4b}$$

where the function $s\,(T_s)$ is:

$$s\,(T_s) = \exp\,(\beta_{Ts}\,(T_s - \mu_{Ts}))/(1 + \exp\,(\beta_{Ts}\,(T_s - \mu_{Ts}))) \tag{11.5}$$

This formulation means that the dormancy-inducing rate I, increases from 0 at low temperatures to K_I at high temperatures. In contrast the dormancy-releasing rate R, decreases from K_R at low temperatures to 0 at high temperatures. The position and rapidity of these changes are controlled by the parameters μ_{Ts} and β_{Ts} respectively, as illustrated in Figure 11.1.

Alternative rate functions were considered that included a reduction in the rate of dormancy release at low temperatures and a decrease in the rate of dormancy induction at high temperatures. Although these were considered to be more physiologically realistic, model fitting did not support the inclusion of the additional parameters required.

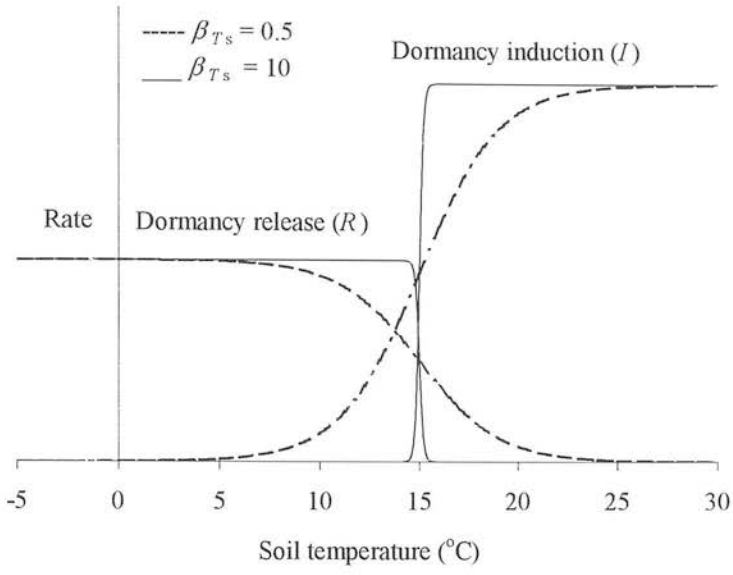


Figure 11.1: Temperature dependence of rates of dormancy induction and release, with illustration of effect of change in parameter β_{Ts} for μ_{Ts} = 15 °C.

With this model and a soil temperature series (Figure 5.5), solving equation (11.3) produces seasonal changes in seed dormancy, with a non-dormant fraction $q(t_d)$ at time t_d.

The probability that a seed will germinate is a function of both the probability that a seed is 'non-dormant' $q(t_d)$ and the environmental conditions. In this model the environmental conditions considered are temperature and water potential. However it should be noted that this model framework could readily be developed to incorporate additional factors, such as light and soil nitrate.

The effect of temperature is modelled by:

$$f(T) = \exp\left(-\left(T - T_{opt}\right)^2 / 2\sigma_T{}^2\right) \qquad (11.6)$$

which represents the probability that a 'non-dormant' seed will germinate in water at a given temperature. The parameter T_{opt} defines the optimal temperature for germination in water and σ_T defines the width of that optimum, as illustrated in Figure 11.2.

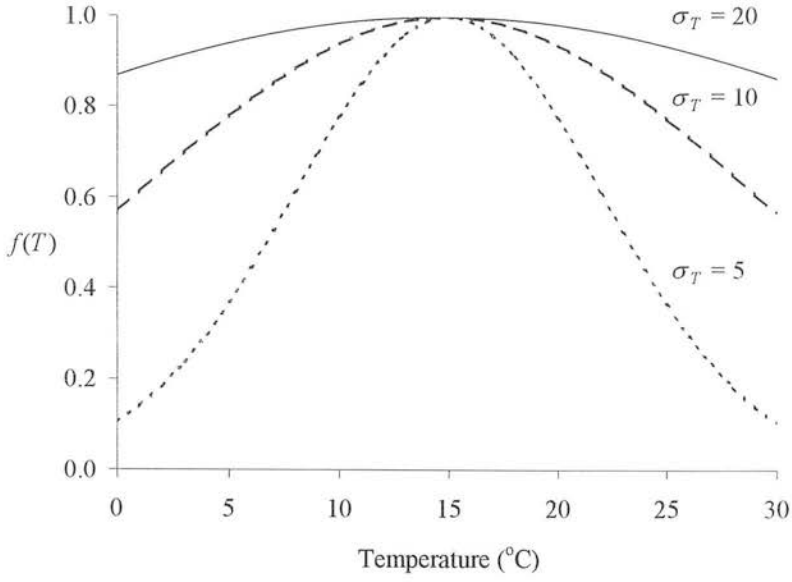


Figure 11.2: $f(T)$ with illustration of changes in parameter σ_T for T_{opt} = 15 °C.

Alternative functions were considered that included an effect of dormancy on the width of the temperature optimum. This is achieved by modifying equation (11.6) by replacing σ_T with $\sigma_T\, q(t_d)$. This corresponds to the idea that increasing seed dormancy narrows the range of temperatures at which seeds germinate (Vleeshouwers, 1997). However model fitting showed that the original version of equation (11.6) was preferable.

The effect of water potential is given by:

$$g(\psi) = \exp(a_{\psi T}\ \psi\ T) \qquad (11.7)$$

which is the probability at temperatures equal to T_{opt}, that a 'non-dormant' seed will germinate for a given water potential. The parameter $a_{\psi T}$ defines the rate at which germination decreases with decreasing water potential and increasing temperature, with more severe effects recorded for larger values of $a_{\psi T}$. The behaviour of $g(\psi)$ is illustrated in Figure 11.3.

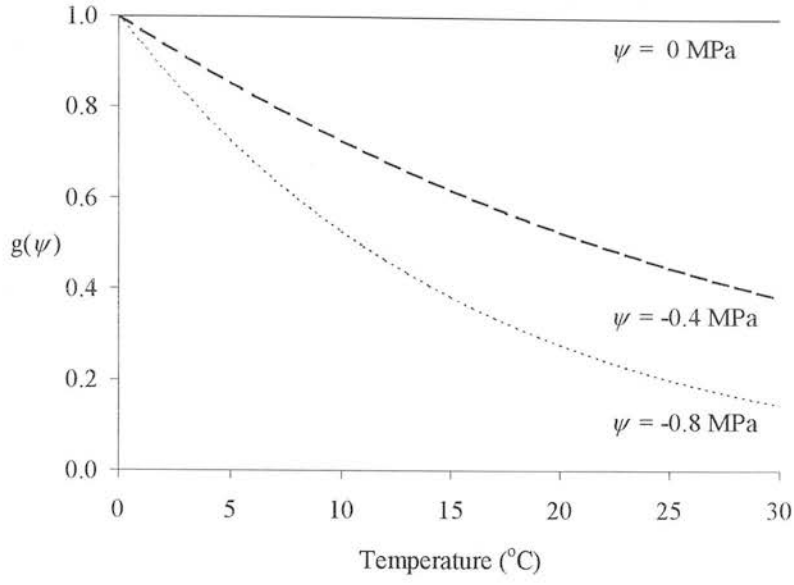


Figure 11.3: $g(\psi)$ for different values of ψ, with $a_{\psi T} = 0.08$

A simpler function for $g(\psi)$, without the temperature and water potential interaction was considered, but model fits were better with equation (11.7)

The environmental effects on 'non-dormant' seeds are summarised by $f(T)\ g(\psi)$ which is the probability that a 'non-dormant' seed is germinable at temperature T and water potential ψ. The probability that a given seed is germinable is then defined as:

$$p\,(\text{germinable}) = q(t_d)\, f(T)\, g(\psi) \qquad (11.8)$$

It is important to realise that this equation does not describe the timing of germination. It can only be used to define the final expected proportion of seeds germinated. As noted, it is assumed that p (germinable) is assumed to remain constant during the germination period.

11.3.2. Timing of germination

Estimation of the proportion of germinable seeds is one part of the problem of predicting weed seedling emergence. Another crucial element is the timing and spread of seed germination. The model described in equation (11.8) is now used as a starting point to develop a model to describe the timing of seed germination. As discussed above, this

stochastic model was formulated in terms of individual seeds, assuming that germination events are independent.

Previously, in section 11.3.1, seasonal changes in seed dormancy were considered, but here for simplicity, dormancy will be considered fixed and as such the model describes the timing of seed germination at a particular point in the dormancy cycle. Such points may be defined by the timing of soil cultivations and associated germination stimuli. This redefines equation (11.8) as:

$$p(\text{germinable}) = q_0 \; f(T)\, g(\psi) \qquad (11.9)$$

where the 'non-dormant' fraction, q_0 is now the only dormancy related parameter to be estimated.

In order to model the timing of seed germination, the probability that the seed germinates during a *small* time interval $(t, t + \delta t)$, following a germination stimulus at $t = 0$, and given that the seed is germinable at time t, p (seed germinates in $(t, t + \delta t)$ | germinable at t), is now defined. If the probability of germination was constant over time, then p (seed germinates in $(t, t + \delta t)$ | germinable at t) = $g_0\, \delta t$, where g_0 is the intrinsic germination rate.

However, previous work has identified thermal time (θ_T) as an important factor in determining the timing of seed germination (Garcia-Huidobro, Monteith & Squire, 1982; Spitters, 1989; van der Weide, 1993; Benech-Arnold *et al.* 1990). This is acknowledged, but whereas Chapters 6 and 7 described thermal time as temperature accumulated above a base temperature, a simpler definition is adopted here, whereby if the temperature during germination is constant, thermal time is defined as Tt (temperature x time), where $t = 0$ corresponds to initiation of germination. This has the benefit of reducing the number of parameters required. If temperature varies during germination, thermal time is defined as the integral of $T(t)$. However only constant temperatures are considered and thermal time is therefore incorporated into the model as:

$$p(\text{seed germinates in } (t,\, t+\delta t) \,|\, \text{germinable at } t) = g_0 \; h\,(Tt)\, \delta t \qquad (11.10)$$

where h is a function of thermal time. Two possible definitions of the function h, were considered. In the simplest case, it was assumed that there was a fixed thermal time requirement μ_{Tt}, and h is therefore defined as a step function:

$$h(Tt) = 0 \qquad \text{if } (Tt) < \mu_{Tt} \tag{11.11}$$
$$h(Tt) = 1 \qquad \text{if } (Tt) >= \mu_{Tt}$$

This corresponds to the view that there is a minimal thermal time requirement below which germination will not occur. Alternatively and perhaps more intuitively, the probability of germination can be assumed to vary continuously with thermal time and this can be modelled as a sigmoid function:

$$h(Tt) = \exp(\beta_{Tt}(Tt - \mu_{Tt}))/(1 + \exp(\beta_{Tt}(Tt - \mu_{Tt}))) \tag{11.12}$$

This appears as a smooth step function where β_{Tt} controls the gradient of the step and μ_{Tt} is now defined as the mean thermal time requirement. It should be noted that this method (11.12) also presents fewer difficulties in terms of parameter estimation (see section 11.4). In Chapters 6 & 7, the probit model used to describe seed germination at different temperature and water potentials was shown to only effectively describe the timing of germination.

In addition to the effect of temperature on thermal time, temperature may also affect the intrinsic rate of germination. Assuming that germination progresses faster, close to the optimal temperature, equation (11.12) may be modified to:

$$p(\text{seed germinates in } (t,\ t+\delta t) \mid \text{germinable at } t) = g_0\ f(T)\, h(Tt)\, \delta t \tag{11.13}$$

where $f(T)$ is the temperature function defined in equation (11.6).

In addition to temperature effects, Chapters 6 & 7 showed the importance of water potential in the timing of seed germination and therefore the time dependent part of the model given in equation (11.13) requires further modification. This is achieved by first modifying the sigmoid function (equation (11.12)) to incorporate the effects of water potential. To do this, two modifications of thermal time were considered.

The first option mirrored the definition of hydrothermal time, as detailed in Chapters 6 & 7, in that thermal time (Tt) is multiplied by a linear function of water potential. Hydrothermal time (HT) is then given by:

$$HT = Tt\left(\psi - \psi_B\right) \tag{11.14}$$

where ψ_B can be interpreted as a base water potential, though it should be noted that this is not necessarily equivalent to the base water potential identified in the hydrothermal time models defined by probit analysis. For this reason, the symbol HT is used to distinguish it from the earlier definition of hydrothermal time θ_{HT} (Chapter 6 & 7).

The second definition of hydrothermal time (HT) multiplies thermal time (Tt) by an exponential function of water potential:

$$HT = Tt \exp\left(a_\psi \psi\right) \tag{11.15}$$

where a_ψ determines the effect of reduced water potential, with more severe effects recorded for larger values of a_ψ. This is illustrated in Figure 11.4, and shows that for water potentials less than 0 MPa, hydrothermal time is less than thermal time.

Whilst both options appeared to explain the data adequately, the second approach was preferred for a number of reasons. This included the fact that in pure water ($\psi = 0$), equation (11.15) is guaranteed to coincide with the thermal time model, in contrast to (11.14). However in either case, equation (11.12) is rewritten as:

$$h\left(HT\right) = \frac{\exp\left(\beta_{HT}\left(HT - \mu_{HT}\right)\right)}{\left(1 + \exp\left(\beta_{HT}\left(HT - \mu_{HT}\right)\right)\right)} \tag{11.16}$$

and this is illustrated in Figure 11.5. It is assumed that the intrinsic germination rate is not modified by water potential, although the model could be easily adapted to incorporate this and other effects.

The final model for the timing of germination is summarised as:

$$p(\text{seed germinates in } (t,\ t+\delta t) \mid \text{germinable at } t) = g_0\ f(T)\, h\,(HT)\,\delta t \qquad (11.17)$$

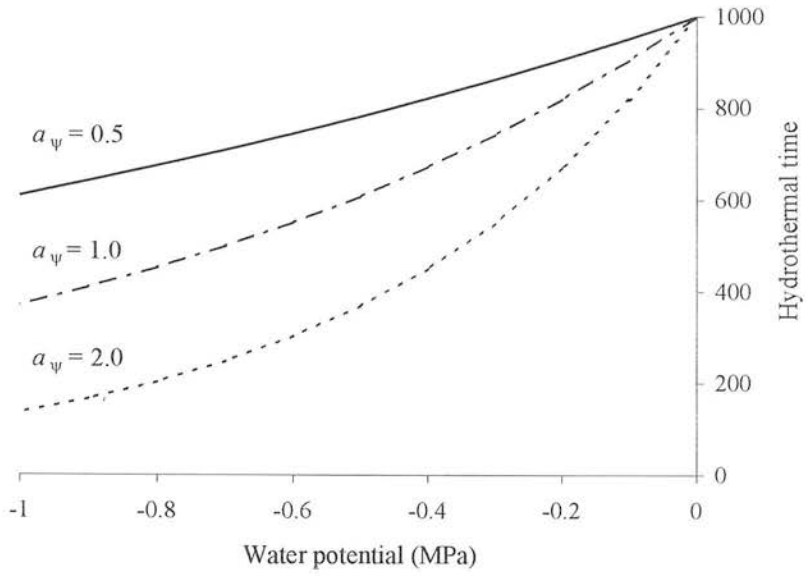


Figure 11.4: Hydrothermal time (*HT*) with illustration of changes in a_ψ for Tt = 1000 °C h.

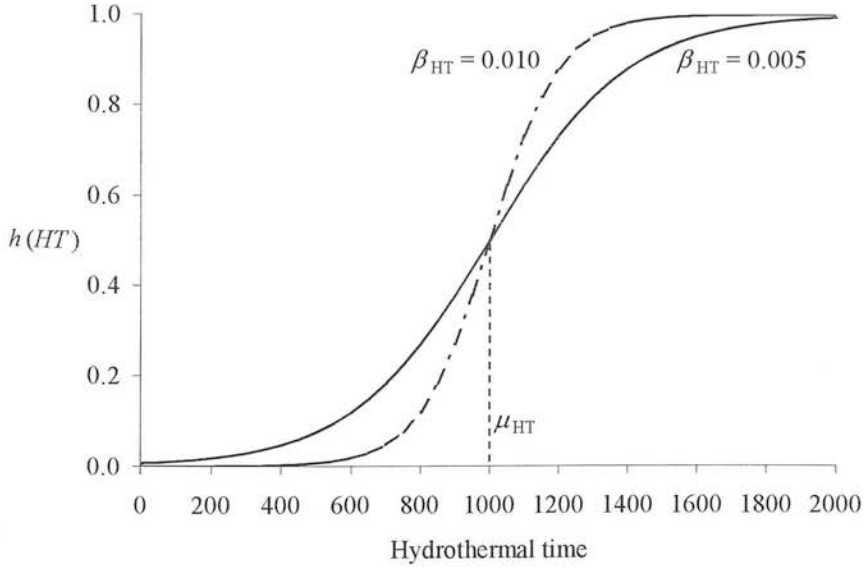


Figure 11.5: $h(HT)$ with illustration of effect of change in β_{HT} for μ_{HT} = 1000.

11.4. Model fitting

Formulation of the model as described in sections 11.3.1 and 11.3.2 allows parameter estimation by maximum likelihood. This is one key advantage of this stochastic model formulation since as described previously (Chapter 2) maximum likelihood provides a statistically sound methodology that allows calculation of standard errors for each estimated parameter. It should be remembered that the likelihood is the probability that a specified model could generate the observed data.

Model fitting used purpose written FORTRAN code and included routines from the NAG library (Numerical Algorithms Group, 1999). Although this was developed and applied by Dr. Glenn Marion, it is included here for completeness.

11.4.1. Fitting the model for seasonal changes in seed dormancy and identification of the germinable seed fraction

To reiterate, this model is concerned with identifying the probability that a seed is germinable. In fitting the model to experimental data it is assumed that the probability that a germinable seed fails to germinate before the end of the experiment is negligible. Therefore the only data required for fitting this model are the final number of seeds germinated and the total number of seeds in the experiment. It is further assumed that the dormancy level remains constant during the experiment. With these assumptions it is particularly straightforward to calculate the likelihood.

The contribution to the likelihood from a germinating seed is:

$$q(t_d)\, f(T)\, g(\psi) \tag{11.18}$$

which is the probability that a seed is germinable (equation (11.8)) at time, t_d in the dormancy cycle.

A seed does not germinate because it is either dormant or 'non-dormant' with conditions for germination not met. The possibility that a seed is not viable is ignored. The contribution to the likelihood from an ungerminated seed is therefore:

$$(1 - q(t_d)) + q(t_d) \times (1 - f(T)\, g(\psi)) \tag{11.19}$$

Since each seed is considered to act independently, the overall likelihood is calculated by multiplying together the individual contributions from each of the seeds. Typically the likelihood for a given set of data is a very small number and therefore it is easier to consider the log of the likelihood (L) which is a function of all the defined parameters:

$$L\left(q_0, k_{\mathrm{I}}, k_{\mathrm{R}}, \beta_{Ts}, \mu_{Ts}, T_{\mathrm{opt}}, \sigma_T, a_{\psi \mathrm{T}}\right) \tag{11.20}$$

Parameter estimates are then obtained by maximising the log likelihood L. Confidence intervals are obtained following the profile likelihood method used by Gibson, Gilligan & Kleczkowski (1999).

11.4.2. Fitting the model for timing of germination

Typically from germination experiments, data are available that define for each seed a time interval (t_1, t_2) in which germination occurs. Seeds that do not germinate are identified with t_{end}, representing the end of observations. Also associated with each seed are stated environmental conditions, namely temperature, T, and water potential ψ.

From equation (11.17) it can be shown that if a seed is germinable, and remains ungerminated at time t_1, then the probability that it remains ungerminated at time t_2 is (e.g. Renshaw, 1991):

$$p(t_2 \mid t_1) = \exp\left(-g_0 \, f(T) \int_{t_1}^{t_2} h\left(HT\right) dt\right) \tag{11.21}$$

It should be noted that the integral $\int_{t_1}^{t_2} h\left(HT\right) dt$ can be easily evaluated for the each of the h functions considered previously. For example, if h is defined as in equation (11.15), then:

$$\begin{aligned} \int_{t_1}^{t_2} h\left(HT\right) dt = & \ln\left(1+\exp\left(\beta_{HT}\left(t_1 \, T \exp\left(a_\psi \, \psi\right) - \mu_{HT}\right)\right)\right) / \left(\beta_{HT} T \exp\left(a_\psi \, \psi\right)\right) \\ & - \ln\left(1+\exp\left(\beta_{HT}\left(t_2 \, T \exp\left(a_\psi \, \psi\right) - \mu_{HT}\right)\right)\right) / \left(\beta_{HT} T \exp\left(a_\psi \, \psi\right)\right) \end{aligned} \tag{11.22}$$

It then follows that if a seed is germinable and remains ungerminated at time t_1, then the probability that it germinates between time t_1 and time t_2 is 1- p ($t_2 \mid t_1$).

The contribution to the likelihood from a germinating seed is then:

$$q_0 \, f(T) \times g(\psi) \times p\,(t_1 \mid t = 0) \times (1 - p\,(t_2 \mid t_1)) \tag{11.23}$$

which can be interpreted as the probability that the seed is germinable multiplied by the probability that it survives to time t_1 multiplied by the probability that it germinates between times t_1 and t_2.

If a seed fails to germinate this can be for three reasons, namely that the seed is dormant, that the seed is not dormant but conditions are unsuited to germination (ungerminable) or that the seed is germinable, but has failed to germinate within the time course of the experiment. This final cause would generally be expected to have a low probability. The contribution to the likelihood from an ungerminated seed is then:

$$(1 - q_0) + q_0\,(1 - f(T)\,g(\psi)) + q_0\, f(T)\,g(\psi) \times p\,(t_{\text{end}} \mid t = 0) \tag{11.24}$$

It is noted that the final term should be multiplied by the probability that the germinable seed (having survived to t_{end}) germinates at some later time. However this probability ($1 - p\,(t \rightarrow \infty \mid t_{\text{end}})$) = 1.

As each seed is considered to act independently, the overall likelihood is calculated by multiplying together the individual contributions from each of the seeds. This is calculated as the log of the likelihood (L), which is a function of all the defined parameters:

$$L\left(q_0, T_{\text{opt}}, \sigma_{\text{T}}, a_{\psi\text{T}}, g_0, \beta_{HT}, \mu_{HT}, a_{\psi}\right) \tag{11.25}$$

Parameter estimates are then obtained by maximising the log likelihood L with respect to all the defined parameters. Confidence intervals are obtained following the profile likelihood method used by Gibson *et al.* (1999).

11.5. Model implementation

11.5.1. Model for seasonal changes in seed dormancy and identification of the germinable fraction

The dormancy model is relatively easy to implement as equations (11.3), (11.4) and (11.5) can be solved for given parameter values in Modelmaker ver. 3.0.3. and other simulation software. This defines the probability that a seed is 'non-dormant' at a given time ($q(t_d)$). Equation (11.8) then calculates the probability that a seed is germinable in a given set of environmental conditions.

If there are n seeds and assuming that individual seeds behave independently, the proportion of germinable seeds follows a binomial distribution where the proportion of germinable seeds at time t is p(germinable) and the variance can be calculated as:

$$\sigma^2 = p\,(\text{germinable})\,\big(1 - p\,(\text{germinable})\big)/n \qquad (11.26)$$

If n is large, the Normal approximation to the binomial distribution allows the upper and lower 95 % confidence intervals to be estimated by p(germinable) $\pm$ 1.96 σ.

This model can be readily implemented in Modelmaker ver. 3.0.3. and other simulation software. This is illustrated Figure 11.6 with a full listing of the associated model definition given in Appendix 2.

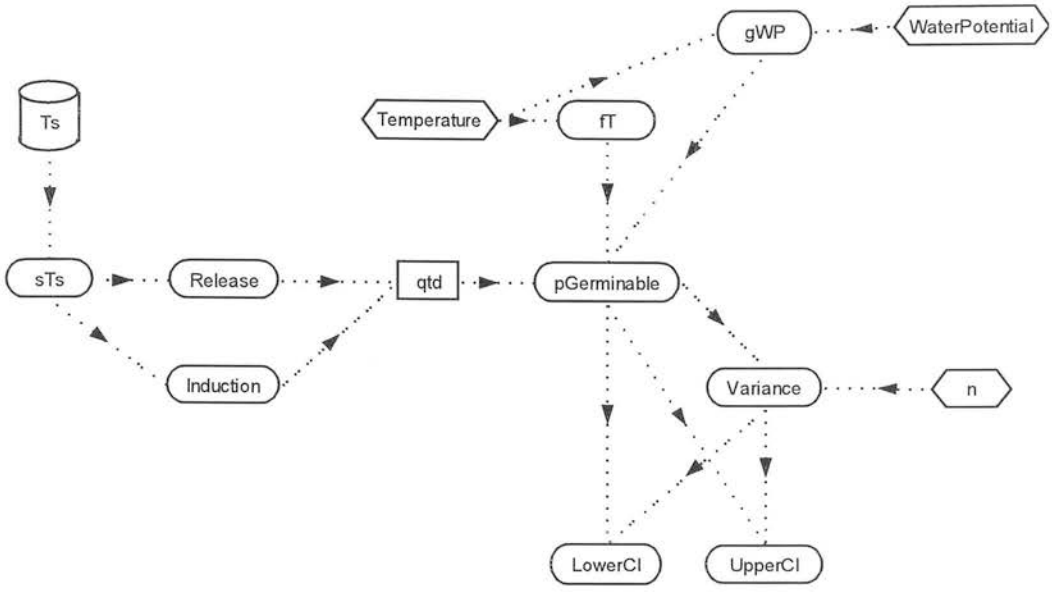


Figure 11.6: Modelmaker ver. 3.0.3. diagram of the dormancy model described in section 11.5.1. Compartment names follow text where possible, with Ts as soil temperature at 10 cm. The function sTs corresponds to equation (11.5). Release defines the rate of dormancy release and Induction defines the rate of dormancy induction according to equation (11.4). qtd is the probability that a seed is not dormant at time t, as defined by equation (11.3). Temperature is the test temperature and WaterPotential is the test water potential. The function fT corresponds to equation (11.6) and gWP to equation (11.7). pGerminable is the probability that a seed is germinable in given test conditions, according to equation (11.8). Variance as equation (11.26) is the variance in the proportion of seeds that are germinable where n is the number of seeds in the test and the upper and lower confidence intervals are UpperCI and LowerCI. Full model definition given in Appendix 2.

11.5.2. Model for timing of germination

The model is easiest to formulate in terms of the probability of a germinable seed remaining ungerminated $p(t \mid 0)$ at time t, in a given set of environmental conditions. Initially at t=0, $p(t \mid 0) = 1$ and subsequent change in $p(t \mid 0)$ is described by:

$$dp\left(t \mid 0\right)/dt = -g_0 \; f(T)\, h(HT)\, p\left(t \mid 0\right) \qquad (11.27)$$

The probability of a germinable seed having germinated by time t is then 1 - $p(t \mid 0)$.

Since the probability that a seed is germinable is given by equation (11.8), the probability that a seed has germinated by time t is:

$$pG = q_0 \; f(T)\, g(\psi) \left(1 - p\left(t \mid 0\right)\right) \tag{11.28}$$

If there are n seeds and assuming that individual seeds behave independently, the number of seeds germinated follows a binomial distribution where the expected number of germinated seeds (G) at time t is:

$$G = n \; pG \tag{11.29}$$

and the variance can be calculated as:

$$\sigma_G^{\,2} = n \; pG \left(1 - pG\right) \tag{11.30}$$

If n is large, the Normal approximation to the binomial distribution allows the upper and lower 95 % confidence intervals to be estimated by ($G \pm 1.96 \; \sigma_G$).

This model can be readily implemented in Modelmaker ver. 3.0.3. and other simulation software. This is illustrated Figure 11.7 with a full listing of the associated model definition given in Appendix 2.

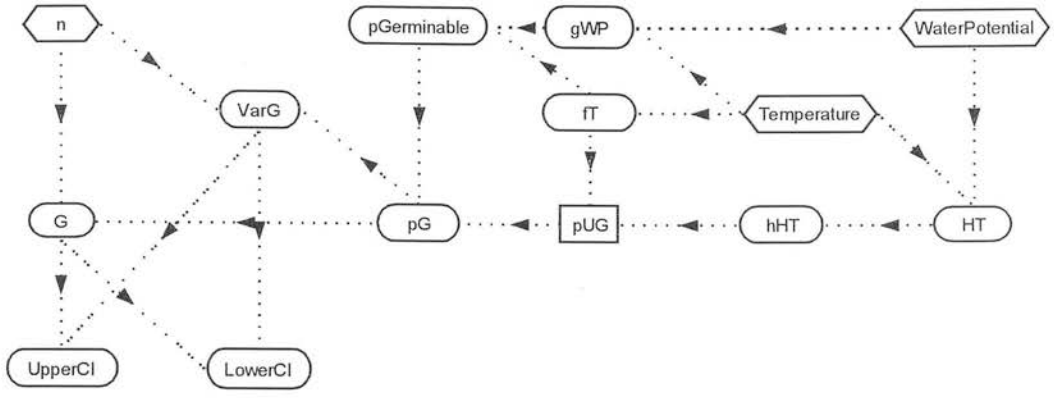


Figure 11.7: Modelmaker ver. 3.0.3. diagram of the germination model described in section 11.5. Compartment names follow text where possible, with Temperature as the test temperature and WaterPotential as the test water potential. HT is the hydrothermal time defined as equation (11.15). The function fT corresponds to equation (11.6), gWP to equation (11.7), hHT to equation (11.16). pGerminable is the probability that a seed is germinable in given test conditions, according to equation (11.8). pUG is the probability of a germinable seed remaining ungerminated at time t, as defined by equation (11.27). pG is the probability that a seed germinates at time t, as defined by equation (11.28). n is the number of seeds in the germination test and G is the expected number of these seeds germinating at time t (assuming individual seeds germinate independently) as equation (11.29). VarG is the variance in number of seeds germinating at time t, as equation (11.30) and the upper and lower confidence intervals are UpperCI and LowerCI. Full model definition given in Appendix 2.

11.6. Model results

The following sections describe the results of fitting the models developed in section 11.3 to seed dormancy and germination data. It should be noted that all the available data was used to fit the models and no independent model validation is presented. As such r^2 statistics describe model goodness of fit and are likely biased estimates of the models predictive ability.

11.6.1. Model for seasonal changes in seed dormancy and identification of the germinable fraction in *Stellaria media*

The model defined in section 11.5.1 was first fitted to the Leicestershire population. Table 11.1 gives the parameters for the fitted model and Figure 11.8 shows the fit of the defined model to data for seasonal changes in the germinable fraction of the Leicestershire population. The graphs are presented with mean predictions and lines for the upper and

lower 95 % confidence intervals. This germinable fraction was determined in monthly germination tests conducted at a range of temperatures and with an initial exposure to red light (soil temperature and germination data as presented in Chapter 5). The model gave a good fit to the data ($r^2 = 0.76$), predicting both initial release of seed dormancy in the autumn and dormancy induction in July. Subsequent dormancy release in the autumn was also predicted, and although some of the variability in this process is reproduced, the observed fit to the data during this period was relatively poor (Figure 11.8). The germinable fraction was underestimated at 20 °C, possibly as a consequence of the small proportion of seeds germinable at 30 °C in the Leicestershire population. This reduced the parameter σ_T compared to the other populations. The parameter β_{Ts} was poorly defined.

The same model was applied to the Perthshire population and parameters for the fitted model are given in Table 11.2. Figure 11.8 shows that this model gave a good fit to the data ($r^2 = 0.93$). However initial dormancy release was poorly represented, possibly because the initial proportion of germinable seeds was high, especially at temperatures between 5 and 20 °C. Dormancy induction in July and subsequent dormancy release in the autumn were predicted, though again the fit to the autumn data was poor. The proportion of germinable seeds at 30 °C was relatively poorly fitted, though this was likely explained by greater variability at this temperature, as represented in wider confidence intervals. The parameters K_I and β_{Ts} were poorly defined.

The same original model was applied to the Caithness population, and again gave a good fit to the data ($r^2 = 0.94$). Table 11.3 gives the parameters for the model and Figure 11.8 shows the fitted model compared with data for seasonal changes in the germinable fraction. Initial dormancy release was poorly represented, though this was again associated with an initial high proportion of 'non-dormant' seeds (q_0). Similarly dormancy induction in July and subsequent dormancy release in the autumn were predicted, though the fit to the autumn data was again poor. The confidence intervals for the proportion of germinable seeds at 30 °C were relatively wide to represent greater variability at this temperature. The parameters K_I and β_{Ts} were also poorly defined.

For all three populations temperature optima (T_{opt}) were similar at approximately 10 °C and the intrinsic rates of dormancy induction (K_I) were greater than those for dormancy release (K_R). This difference was particularly marked for the Perthshire and Caithness populations and this was associated with abrupt dormancy induction.

Parameter	Value	95 % confidence interval	
q_0	0.52	0.44	0.61
K_I	33.75	24.94	46.45
K_R	8.88	7.16	11.05
β_{Ts}	50.00	3.90	50.00
μ_{Ts}	14.01	13.71	14.26
T_{opt}	10.30	9.20	11.14
σ_T	10.58	10.41	11.61

Table 11.1: Parameter values with associated confidence intervals for the fitted dormancy model for the Leicestershire population of *Stellaria media*. Model defined as in section 11.5.2. $r^2 = 0.76$.

Parameter	Value	95 % confidence interval	
q_0	0.91	0.85	0.95
K_I	12966.16	61.44	100000.00
K_R	6.37	0.77	8.94
β_{Ts}	14.00	1.96	50.00
μ_{Ts}	15.21	14.28	18.78
T_{opt}	11.21	8.91	13.00
σ_T	20.84	17.81	25.09

Table 11.2: Parameter values with associated confidence intervals for the fitted dormancy model for the Perthshire population of *Stellaria media*. Model defined as in section 11.5.2. $r^2 = 0.93$.

Parameter	Value	95 % confidence interval	
q_0	0.90	0.86	0.94
K_I	1373.80	76.63	10000.00
K_R	2.54	1.54	3.63
β_{Ts}	50.00	2.36	50.00
μ_{Ts}	15.11	14.10	17.22
T_{opt}	10.60	7.17	13.01
σ_T	29.46	24.24	37.67

Table 11.3: Parameter values with associated confidence intervals for the fitted dormancy model for the Caithness population of *Stellaria media*. Model defined as in section 11.5.2. $r^2 = 0.94$.

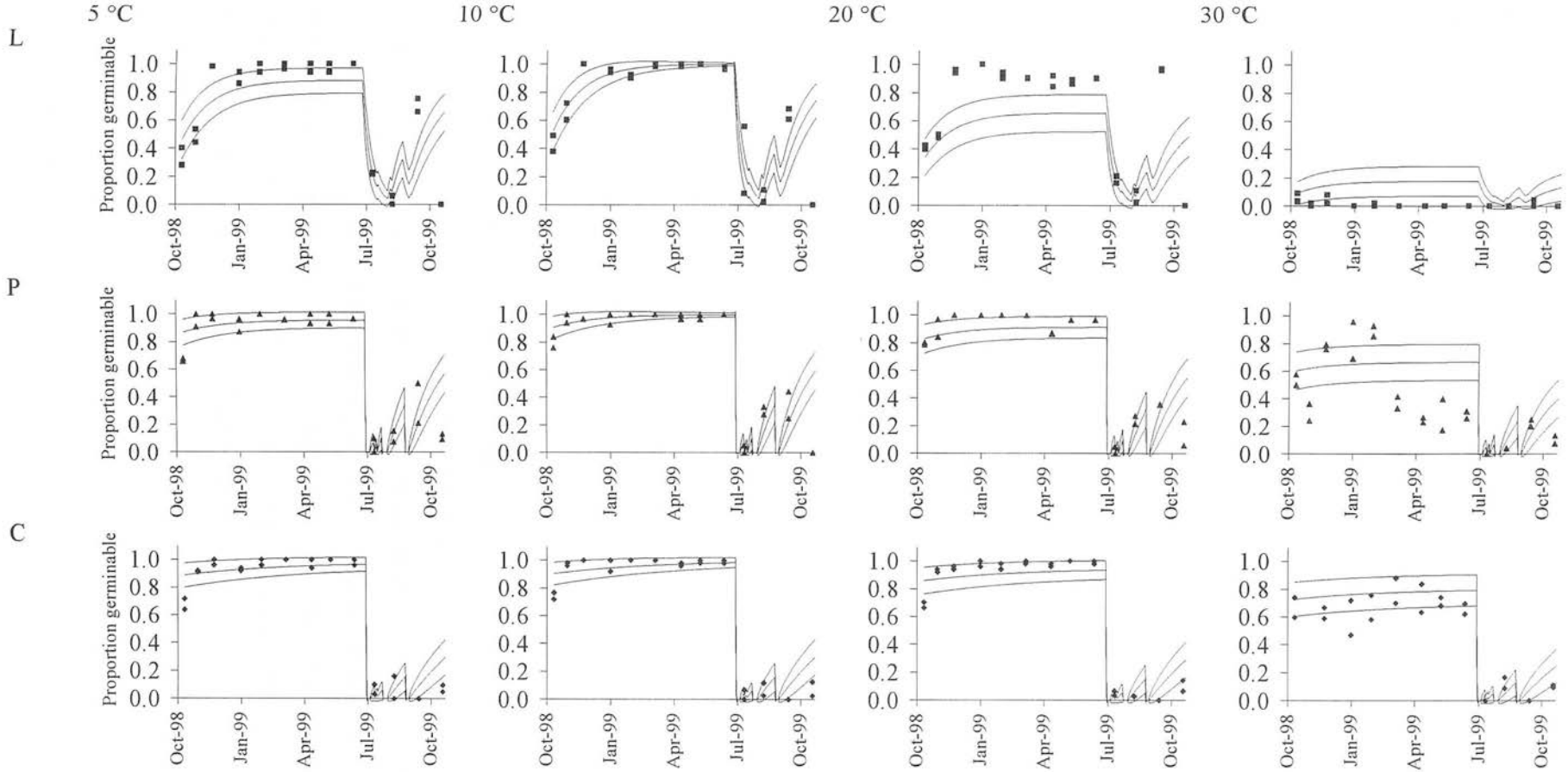


Figure 11.8: Fitted model compared with data for seasonal changes in the proportion of germinable seeds at constant test temperatures for populations of *Stellaria media*. Data are from Block 2: n = 2. Model defined as in section 11.5.1 and presented with mean and 95 % confidence intervals.
Population codes: L: Leicestershire; P: Perthshire; C: Caithness.

11.6.2. Model for timing of germination in *Stellaria media*

The model defined in section 11.5.2 was first fitted to the Leicestershire population. Table 11.4 gives the parameters for the fitted model and Figure 11.9 shows the fit of the defined model to data for germination of the Leicestershire population at a range of temperatures and water potentials following a 15 minute exposure to red light (data as presented in Chapter 6). The model gives a good fit to the data ($r^2 = 0.85$), predicting both the timing of germination and final percentage germination in each temperature and water potential combination, although final percentage germination was overestimated at 5 °C in –0.8 MPa.

The same model was applied to the Perthshire population and parameters for the fitted model are given in Table 11.5. Although model fit was still good ($r^2 = 0.85$), Figure 11.10 shows that although the model made good predictions for the timing of germination, predictions of final percentage germination were less successful. Final percentage germination was notably overestimated at 5 °C in –0.8 MPa and underestimated at 5 °C in distilled water (0 MPa) and 10 and 20 °C in –0.4 MPa. The model was therefore adapted and the effect of temperature on final percentage germination and the intrinsic rate of germination were removed. From observation, this gave a better fit to seed germination at 0 MPa, although the model continued to overestimate final percentage germination at 5 °C in –0.8 MPa and underestimate final percentage germination at 10 and 20 °C in –0.4 and –0.8 MPa. As such, overall model fit was not significantly different ($r^2 = 0.83$). The parameter estimates of the adapted model are given in Table 11.6 and the fit of the model to experimental data is given in Figure 11.11.

The same original model (as defined in section 11.5.2) was applied to the Caithness population, but gave a poorer fit to the data (Table 11.7, $r^2 = 0.62$). This was associated with failure of the model fitting procedure to define the parameters T_{opt} and σ_T. This failure likely reflected the surprisingly high levels of germination recorded in this population at 30 °C. Two options for improving the fit of the model to the data were considered. These were to either to adapt the model by removing the effect of temperature on final percentage germination and the intrinsic rate of germination or to restrict the fitting procedure to germination at 5, 10 and 20 °C only. Table 11.8 gives the parameters for model with effect of temperature on final percentage germination and the intrinsic rate of germination were removed and Table 11.9 gives the parameters for the model fitted to germination at temperatures less than 30 °C. For the model without temperature effects, the timing of

germination was well described by the model (Figure 11.12) but final percentage germination was consistently overestimated at –0.8 MPa and underestimated at 30 °C. Table 11.9 shows that although the parameters T_{opt} and σ_T remained poorly defined for the model restricted to 5, 10 and 20 °C, the model fit to the data was significantly improved ($r^2 = 0.75$, Figure 11.13).

Parameter	Value	95 % confidence interval	
q_0	0.7240	0.6198	0.8198
g_0	0.0581	0.0444	0.0767
T_{opt}	19.34	17.92	21.18
σ_T	10.94	9.40	13.50
$a_{\psi T}$	0.0754	0.0531	0.0998
μ_{HT}	894.27	828.44	967.49
β_{HT}	0.0147	0.0103	0.0223
a_{ψ}	0.7112	0.5862	0.8406

Table 11.4: Parameter values with associated confidence intervals for the fitted germination model for the Leicestershire population of *Stellaria media* at a range of constant temperatures and water potentials following a 15 min. exposure to red light. Model defined as in section 11.5.2. $r^2 = 0.85$.

Parameter	Value	95 % confidence interval	
q_0	0.9670	0.9248	0.9954
g_0	0.0316	0.0262	0.0383
T_{opt}	19.13	16.84	22.38
σ_T	18.14	14.44	25.00
$a_{\psi T}$	0.0839	0.0674	0.1025
μ_{HT}	884.00	821.66	951.28
β_{HT}	0.0119	0.0091	0.0162
a_{ψ}	0.7422	0.6224	0.8657

Table 11.5: Parameter values with associated confidence intervals for the fitted germination model for the Perthshire population of *Stellaria media* at a range of constant temperatures and water potentials following a 15 min. exposure to red light. Model defined as in section 11.5.2. $r^2 = 0.85$.

Parameter	Value	95 % confidence interval	
q_0	0.8938	0.8485	0.9309
g_0	0.0265	0.0227	0.0309
$a_{\psi T}$	0.0945	0.0789	0.1112
μ_{HT}	888.59	833.46	947.81
β_{HT}	0.0118	0.0093	0.0154
a_ψ	0.7377	0.6324	0.8456

Table 11.6: Parameter values with associated confidence intervals for the fitted germination model for the Perthshire population of *Stellaria media* at a range of constant temperatures and water potentials following a 15 min. exposure to red light. Model adapted from that defined in section 11.5.2 by removal of temperature effect on final percentage germination or the intrinsic rate of germination. $r^2 = 0.83$.

Parameter	Value	95 % confidence interval	
q_0	1.0000	0.2548	1.0000
g_0	0.0702	0.0191	0.1026
T_{opt}	164.82	20.70	>500.00
σ_T	93.45	16.05	388.85
$a_{\psi T}$	0.0225	0	0.0480
μ_{HT}	858.19	735.68	986.11
β_{HT}	0.0097	0.0062	0.0171
a_ψ	1.2298	0.8915	1.6361

Table 11.7: Parameter values with associated confidence intervals for the fitted germination model for the Caithness population of *Stellaria media* at a range of constant temperatures and water potentials following a 15 min. exposure to red light. Model defined as in section 11.5.2. $r^2 = 0.62$.

Parameter	Value	95 % confidence interval	
q_0	0.2609	0.2155	0.3121
g_0	0.0228	0.0180	0.0291
$a_{\psi T}$	0.0114	0	0.0338
μ_{HT}	904.52	801.42	1015.07
β_{HT}	0.0091	0.0063	0.0143
a_ψ	1.1911	0.9137	1.4974

Table 11.8: Parameter values with associated confidence intervals for the fitted germination model for the Caithness population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model adapted from that defined in section 11.5.2 by removal of temperature effect on final percentage germination. $r^2 = 0.51$.

Parameter	Value	95 % confidence interval	
q_0	0.3982	0.2814	0.5543
g_0	0.0319	0.0211	0.1263
T_{opt}	15.92	12.79	372.72
σ_T	10.55	6.67	232.65
$a_{\psi T}$	0.1456	0.0863	0.2140
μ_{HT}	904.68	774.18	1090.76
β_{HT}	0.0102	0.0057	0.0196
a_ψ	0.9897	0.6827	1.3944

Table 11.9: Parameter values with associated confidence intervals for the fitted germination model for the Caithness population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model as defined in section 11.5.2, but fitted to 5, 10 and 20 °C only. $r^2 = 0.75$.

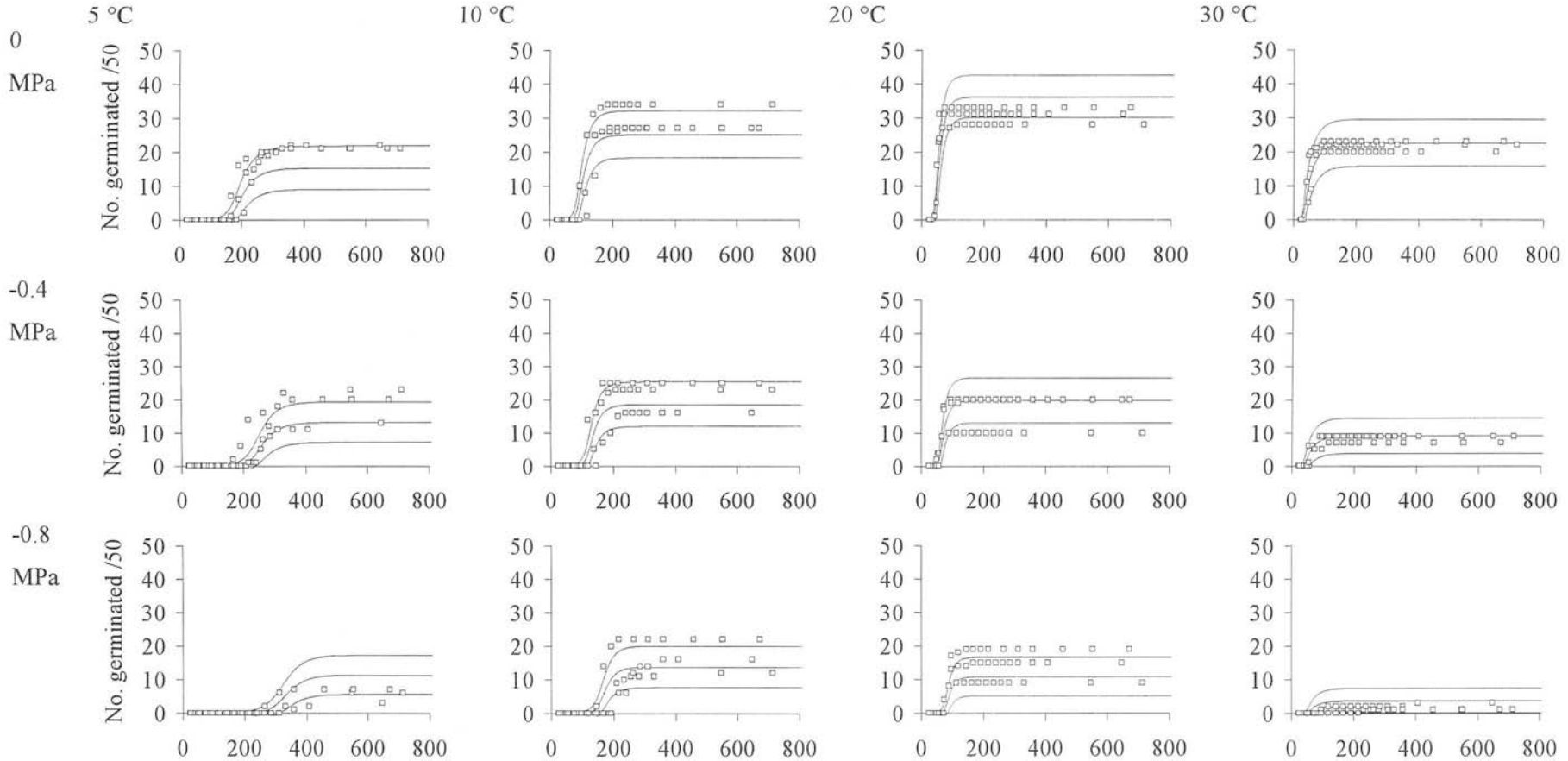


Figure 11.9: Fitted model compared with data for germination of the Leicestershire population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model defined as in section 11.5.2 and presented with mean and 95 % confidence intervals. $r^2 = 0.85$.
n = 3.

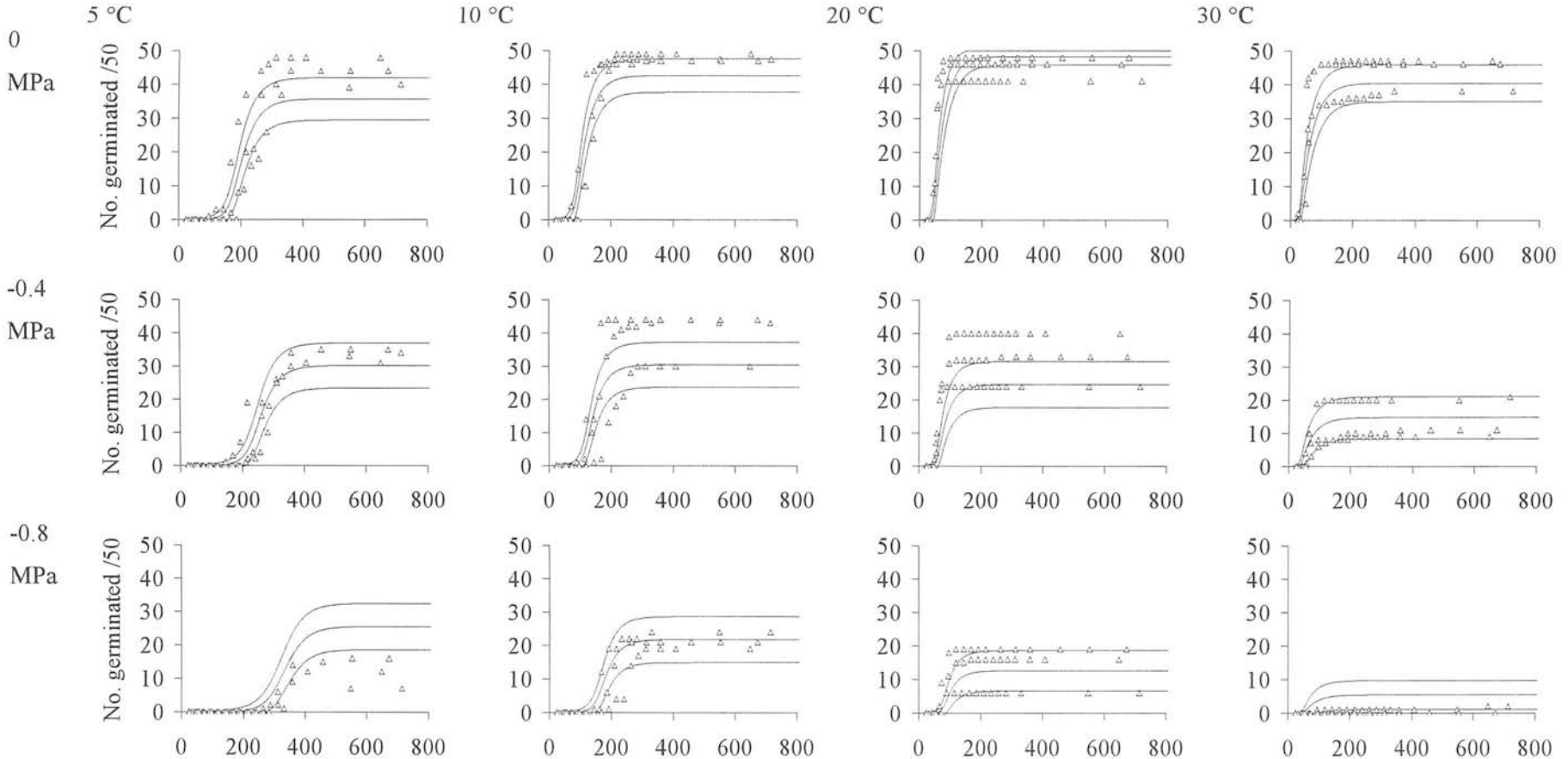


Figure 11.10: Fitted model compared with data for germination of the Perthshire population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model defined as in section 11.5.2 and presented with mean and 95 % confidence intervals. $r^2 = 0.85$.
n = 3.

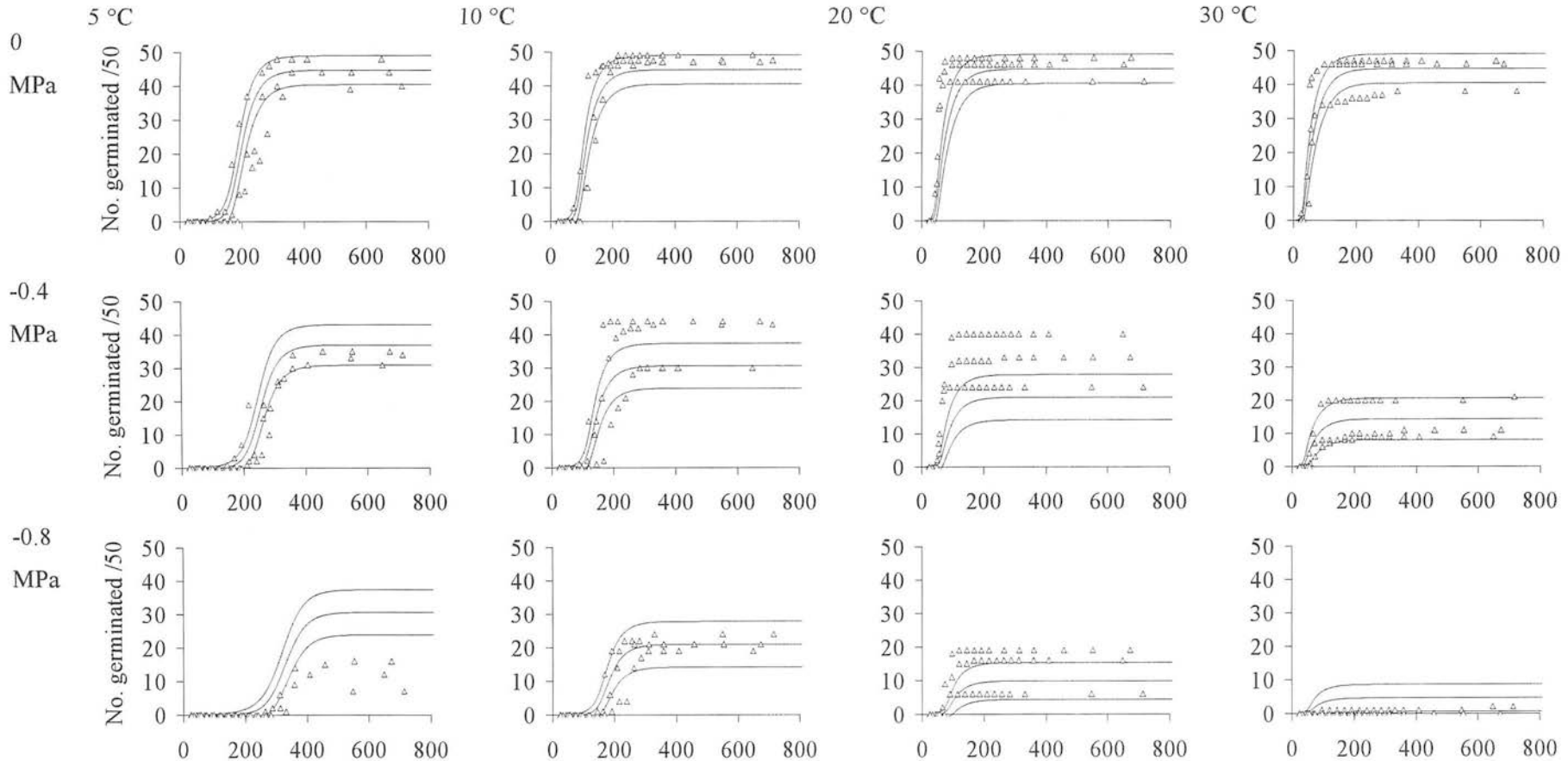


Figure 11.11: Fitted model compared with data for germination of the Perthshire population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model adapted from section 11.5.2 with no temperature effect on final percentage germination or the intrinsic rate of germination. Model presented with mean and 95 % confidence intervals. $r^2 = 0.83$. n = 3.

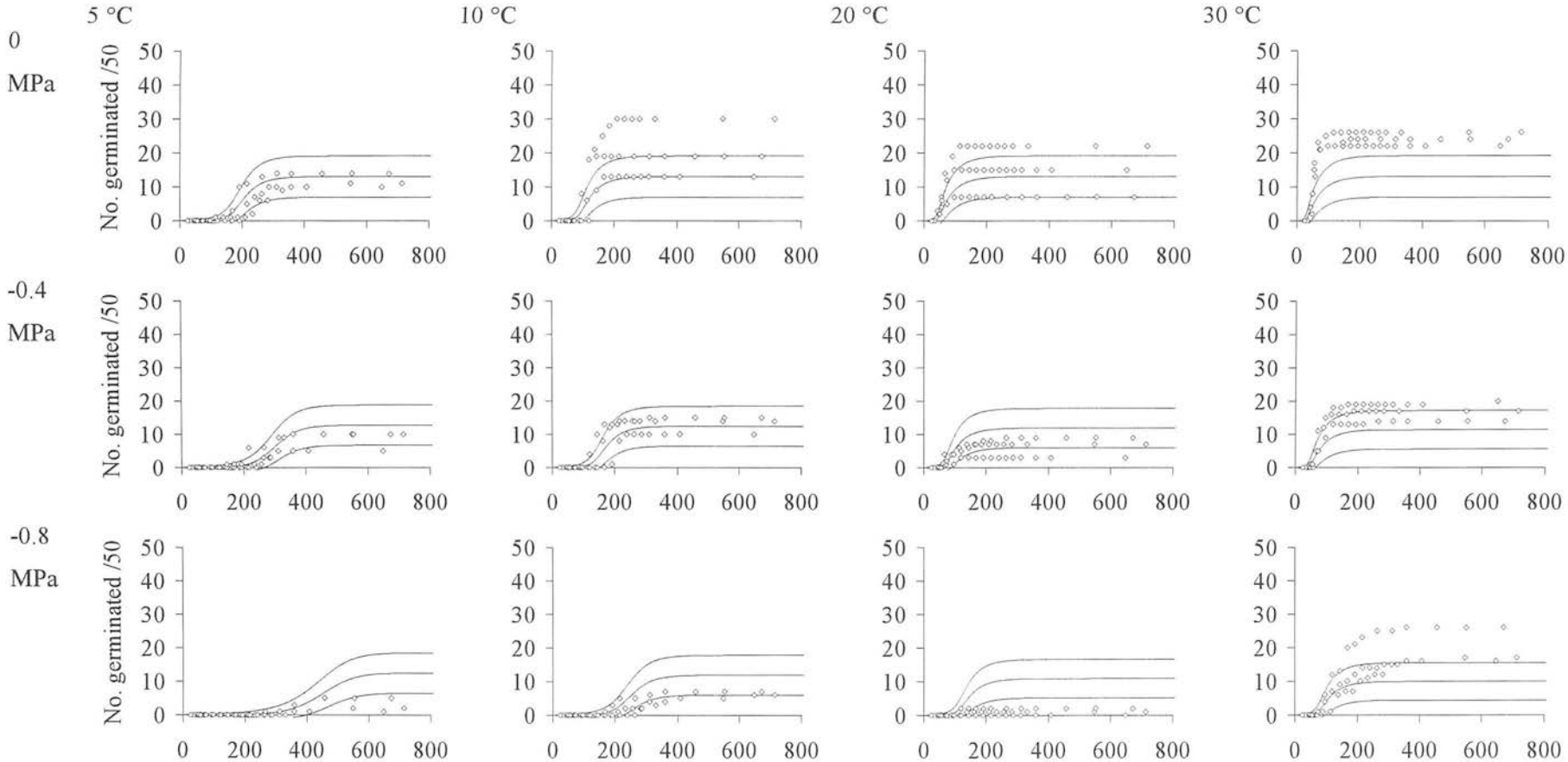


Figure 11.12: Fitted model compared with data for germination of the Caithness population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model adapted from section 11.5.2 with no temperature effect on final percentage germination or the intrinsic rate of germination. Model presented with mean and 95 % confidence intervals. $r^2 = 0.51$. n = 3.

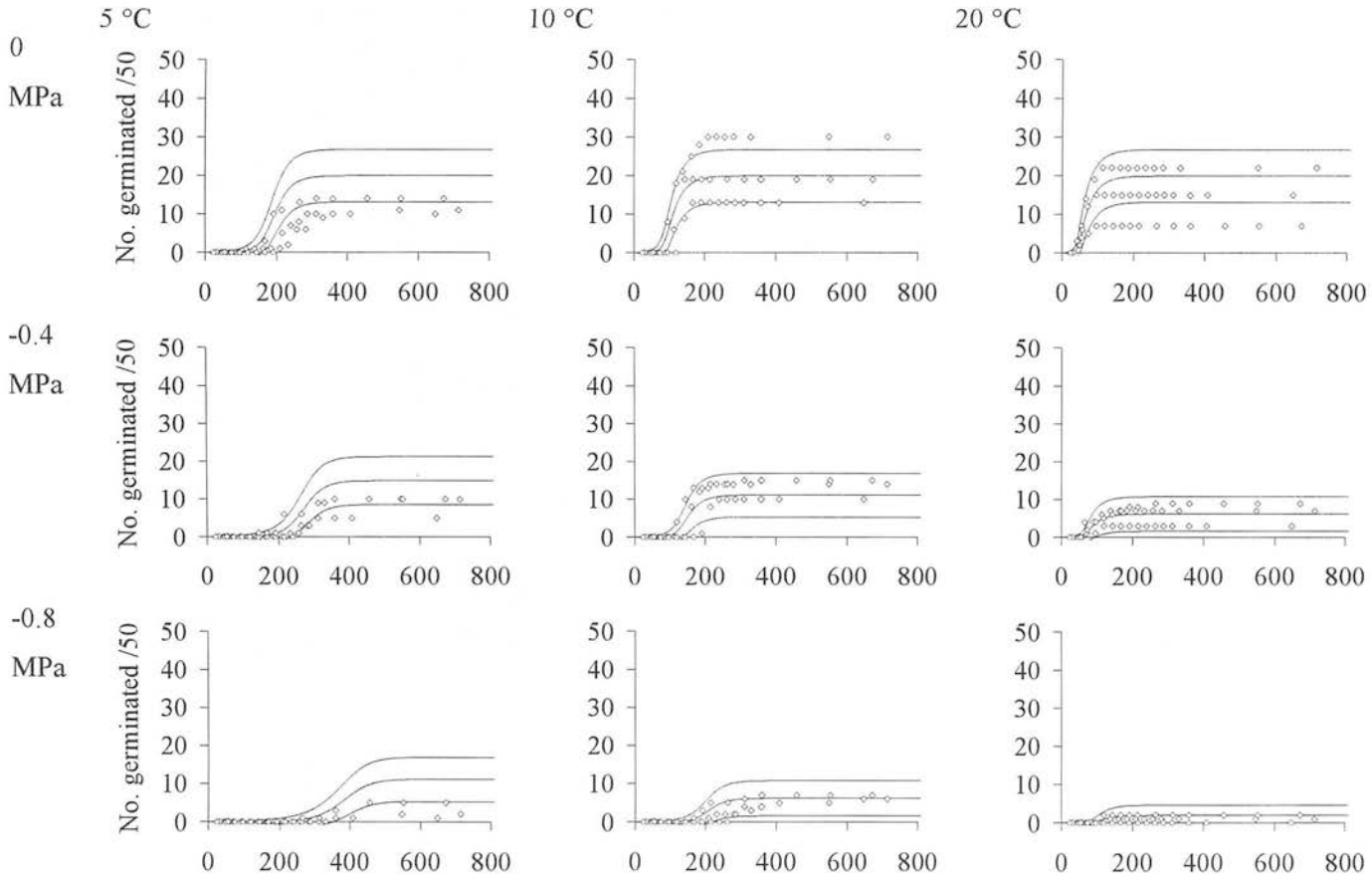


Figure 11.13: Fitted model compared with data for germination of the Caithness population of *Stellaria media* at a range of constant temperatures and water potentials following exposure to red light. Model defined as in section 11.5.2, but fitted to data from 5, 10 and 20 °C only and presented with mean and 95 % confidence intervals. $r^2 = 0.75$.
n = 3.

11.6.3. Model for seasonal changes in seed dormancy and identification of the germinable fraction in *Galium aparine*

The model defined in section 11.5.1 was fitted to data for seasonal changes in *G. aparine* seed dormancy, as presented by van der Weide (1993). Data were available for a single population, but seasonal changes in soil temperature at 10 cm were not presented. Therefore model fitting used the soil temperature series presented in Figure 5.5. Initial seed dormancy was recorded and buried seeds were unearthed on five occasions over a period of 13 months. Germination of unearthed seeds was tested at 11 constant temperatures, with one replicate (further details in section 4.6.4).

Table 11.10 gives the parameters for the fitted model and Figure 11.14 shows the fit of the defined model to data for seasonal changes in the germinable fraction. The graphs are presented with mean predictions and lines for the upper and lower 95 % confidence intervals. The model gave a reasonable fit to the data ($r^2 = 0.57$), predicting both initial release of seed dormancy in the autumn, dormancy induction in the summer and subsequent dormancy release in the second autumn. The model underestimated both the proportion of seeds germinable at 17 and 19 °C over winter and the extent of dormancy induction in the summer. Low levels of germination were also predicted at 0, 21 and 23 °C, despite limited or no recorded seed germination. Table 11.10 shows that the parameters K_I, K_R and $\mu_{\Gamma s}$ were poorly defined.

In an attempt to improve the model fits, an alternative temperature function was considered that included an effect of dormancy on the width of the temperature optimum. This was achieved by modifying equation (11.6) by replacing σ_T with $\sigma_T\, q(t_d)$ and this corresponded to the idea that increasing seed dormancy narrows the range of temperatures at which seeds germinate (Vleeshouwers, 1997). Table 11.11 gives the fitted parameters for this model and Figure 11.15 shows the fit of the defined model to data for seasonal changes in the germinable fraction. There was a marginal improvement in the fit of the model to the data ($r^2 = 0.58$), with better representation of dormancy induction in the summer. However the model continued to underestimate the proportion of seeds germinable at 17 and 19 °C over winter and to predict low levels of germination at 0, 21 and 23 °C. The parameters K_I, K_R and $\mu_{\Gamma s}$ also remained poorly defined.

For both models, temperature optima (T_{opt}) were similar at approximately 12 °C and the temperature optima were relatively narrow, compared with *S. media* (section 11.6.1). The intrinsic rate for dormancy induction (K_I) was also greater than that for dormancy release (K_R).

Parameter	Value	95 % confidence interval	
q_0	0.48	0.31	0.66
K_I	6415.28	10.35	10000.00
K_R	32.20	10.64	100.00
β_{Ts}	0.25	0.12	0.42
μ_{Ts}	34.38	0.72	60.00
T_{opt}	12.06	11.46	12.64
σ_T	4.14	3.70	4.67

Table 11.10: Parameter values with associated confidence intervals for the fitted dormancy model for *G. aparine* (data from van der Weide, 1993). Model defined as in section 11.5.2. $r^2 = 0.57$.

Parameter	Value	95 % confidence interval	
q_0	0.59	0.48	0.71
K_I	4663.77	10.59	10000.00
K_R	38.70	12.89	100.00
β_{Ts}	0.17	0.10	0.27
μ_{Ts}	41.91	28.01	60.00
T_{opt}	11.70	11.12	12.26
σ_T	5.76	4.81	7.08

Table 11.11: Parameter values with associated confidence intervals for the fitted dormancy model for *G. aparine* (data from van der Weide, 1993). Model adapted to incorporate an effect of dormancy on the width of the temperature optimum. $r^2 = 0.58$.

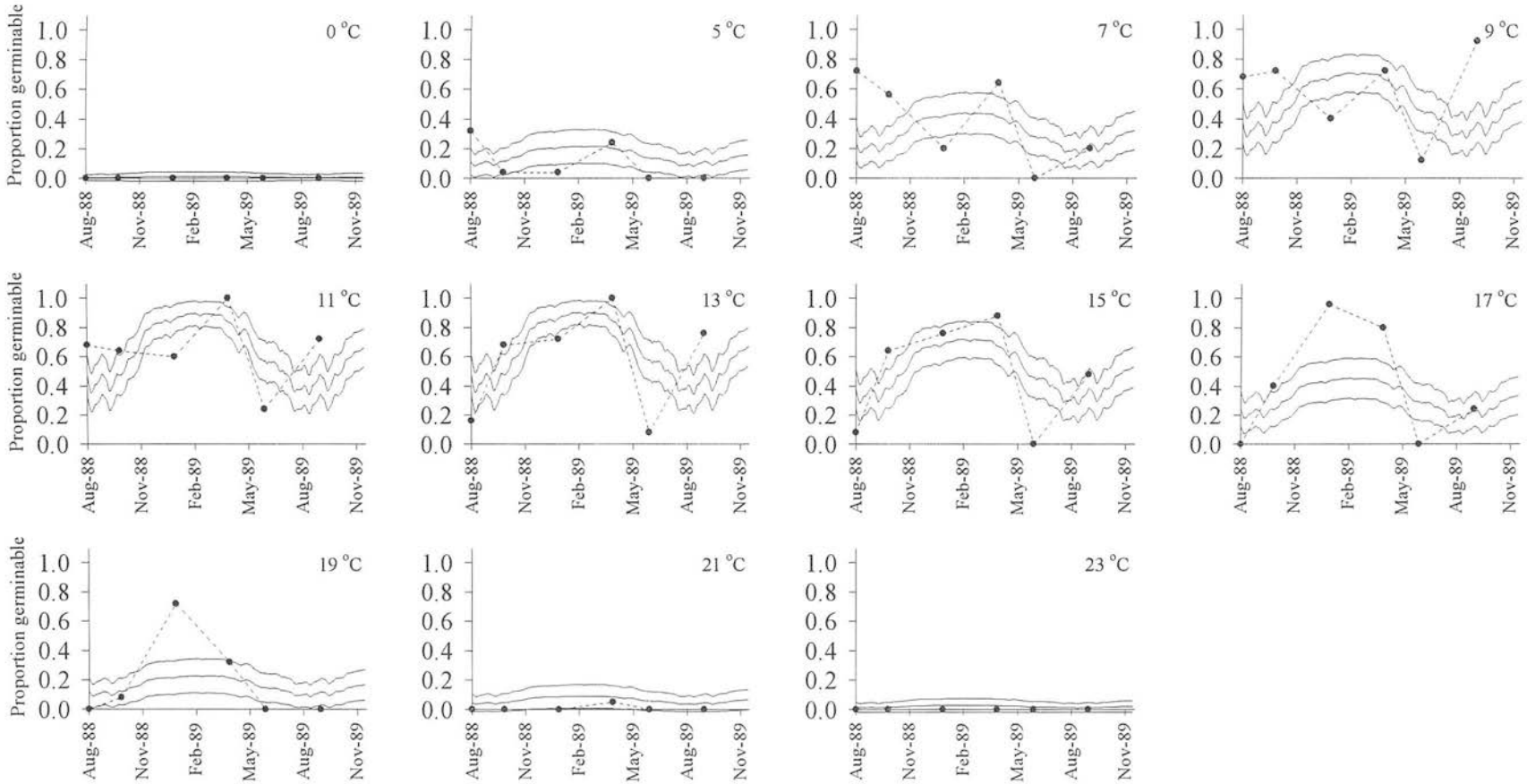


Figure 11.14: Fitted model compared with data for seasonal changes in the proportion of germinable seeds at constant test temperatures for *Galium aparine*. Data are from van der Weide (1993). Model defined as in section 11.5.1 and presented with mean and 95 % confidence intervals. $r^2 = 0.57$. n = 1.

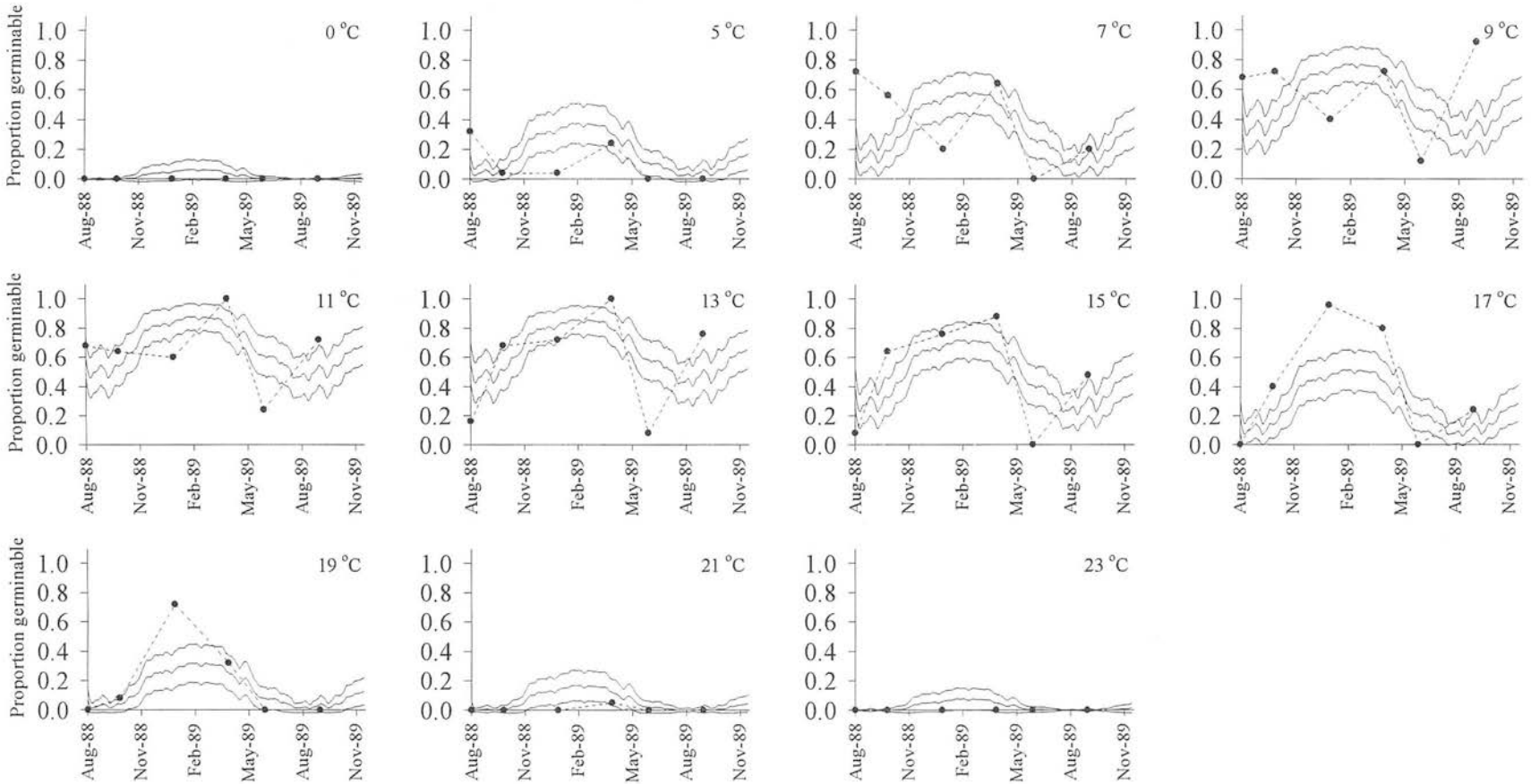


Figure 11.15: Fitted model compared with data for seasonal changes in the proportion of germinable seeds at constant test temperatures for *Galium aparine*. Data are from van der Weide (1993). Model adapted to incorporate an effect of dormancy on the width of the temperature optimum and presented with mean and 95 % confidence intervals. $r^2 = 0.58$. n = 1.

11.6.4. Model for timing of germination in *Galium aparine*

The model defined in section 11.5.2 was first fitted to the North Yorkshire population. Table 11.12 gives the parameters for the fitted model. It should also be noted from Table 11.12 that the parameters T_{opt} and σ_T were poorly defined and therefore a simpler model without temperature effects on final percentage germination or the intrinsic rate of germination was defined. The parameters for this model are given in Table 11.13 and Figure 11.16 shows the fit of this model to germination data for the North Yorkshire population at a range of temperatures and water potentials following a 15 minute exposure to red light (data as presented in Chapter 7). This simpler model gave as good a fit to the data (r^2 = 0.87), predicting both the timing of germination and final percentage germination in each temperature and water potential combination, although final percentage germination was underestimated at 5 and 10 °C in –0.4 MPa.

The model defined in section 11.5.2 was then applied to the Northumberland population. This model gave a good fit to the data (r^2 = 0.88), although the parameter σ_T was poorly defined. The parameter values are given in Table 11.14 and the model fit is shown in Figure 11.17. At 5 and 10 °C the model underestimated the speed of germination in water (0 MPa) and final percentage germination at –0.4 MPa. Conversely the model overestimated final percentage germination at 5 °C in –0.8 MPa.

This same model (as defined in section 11.5.2) was also successfully applied (r^2 = 0.89) to the Northern Ireland population. The parameter values are given in Table 11.15 and the model fit is shown in Figure 11.18. Again at 5 and 10 °C the model tended to underestimate the speed of germination in water (0 MPa) and final percentage germination at –0.4 MPa. Final percentage germination was also underestimated in –0.4 MPa at 30 °C and overestimated in –0.8 MPa at 5 °C.

Parameter	Value	95 % confidence interval	
q_0	0.7526	0.6481	1.0000
g_0	0.0076	0.0059	0.0170
T_{opt}	0	0	445.47
σ_T	499.95	8.15	>500.00
$a_{\psi T}$	0.1225	0.0575	0.1879
μ_{HT}	1099.55	880.83	1448.99
β_{HT}	0.0080	0.0038	0.0169
a_ψ	1.6646	1.2036	2.2354

Table 11.12: Parameter values with associated confidence intervals for the fitted germination model for the North Yorkshire population of *Galium aparine* at a range of constant temperatures and water potentials following exposure to red light. Model as defined in section 11.5.2. $r^2 = 0.87$.

Parameter	Value	95 % confidence interval	
q_0	0.7523	0.6801	0.8170
g_0	0.0076	0.0063	0.0091
$a_{\psi T}$	0.1225	0.0967	0.1543
μ_{HT}	1099.48	980.49	1226.57
β_{HT}	0.0080	0.0056	0.0118
a_ψ	1.6645	1.4066	1.9208

Table 11.13: Parameter values with associated confidence intervals for the fitted germination model for the North Yorkshire population of *Galium aparine* at a range of constant temperatures and water potentials following exposure to red light. Model adapted from that defined in section 11.5.2 by removal of temperature effect on final percentage germination and the intrinsic rate of germination. $r^2 = 0.87$.

Parameter	Value	95 % confidence interval	
q_0	0.9991	0.9677	1.0000
g_0	0.0056	0.0047	0.0067
T_{opt}	7.67	0	12.15
σ_T	25.82	15.31	134.67
$a_{\psi T}$	0.1539	0.1241	0.1885
μ_{HT}	998.58	889.16	1112.54
β_{HT}	0.0120	0.0068	0.0221
a_ψ	1.0433	0.7926	1.3256

Table 11.14: Parameter values with associated confidence intervals for the fitted germination model for the Northumberland population of *Galium aparine* at a range of constant temperatures and water potentials following exposure to red light. Model as defined in section 11.5.2. $r^2 = 0.88$.

Parameter	Value	95 % confidence interval	
q_0	1.0000	0	1.0000
g_0	0.0065	0.0050	0.0095
T_{opt}	7.82	0.95	9.58
σ_T	12.16	9.08	22.47
$a_{\psi T}$	0.1057	0.0684	0.1345
μ_{HT}	1097.50	879.99	1247.75
β_{HT}	0.0059	0.0038	0.0103
a_ψ	2.0076	1.4392	3.0294

Table 11.15: Parameter values with associated confidence intervals for the fitted germination model for the Northern Ireland population of *Galium aparine* at a range of constant temperatures and water potentials following exposure to red light. Model as defined in section 11.5.2. $r^2 = 0.89$.

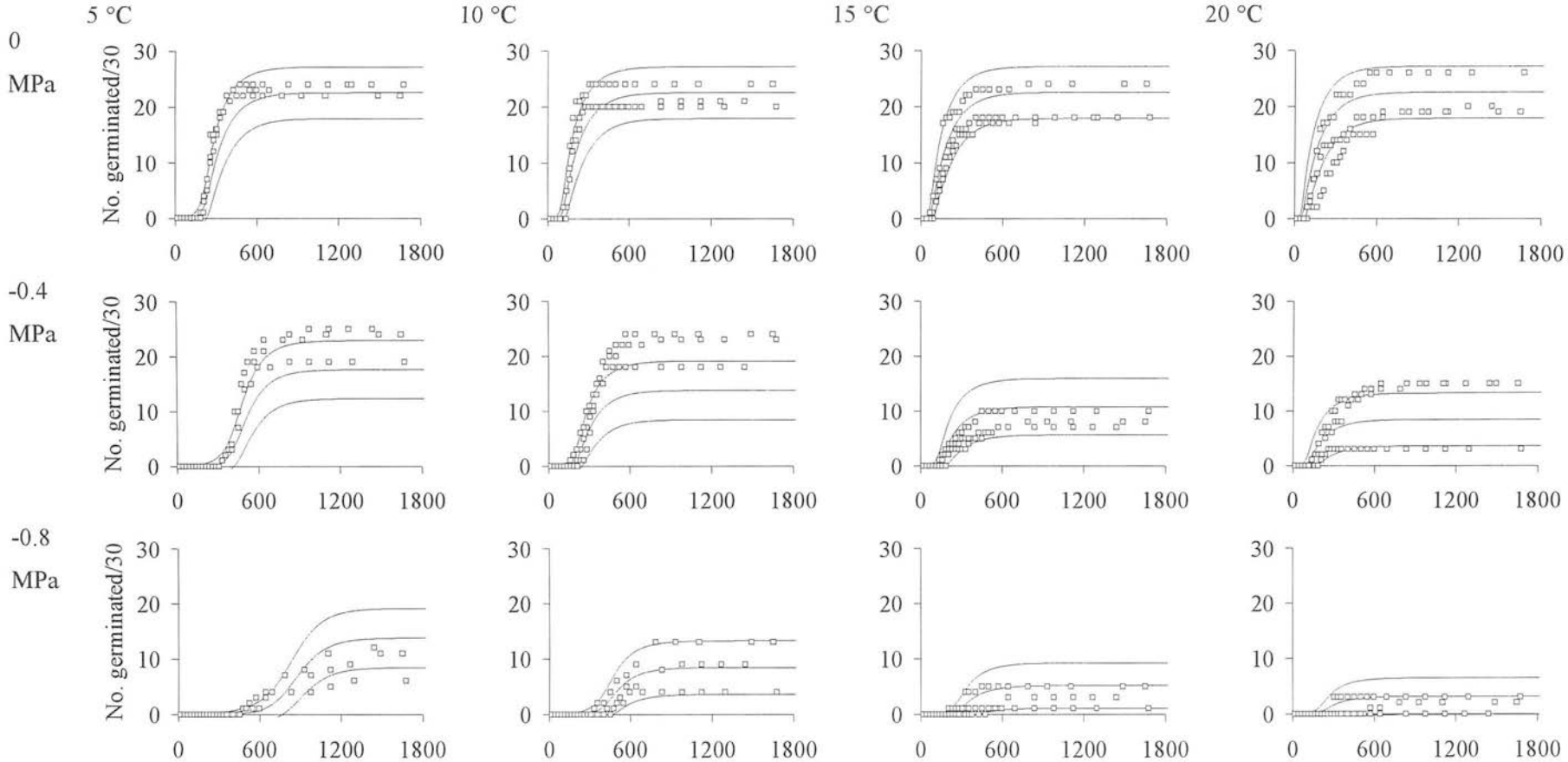


Figure 11.16: Fitted model compared with data for germination of the North Yorkshire population of *Galium aparine* at a range of constant temperatures and water potentials following exposure to red light. Model adapted from section 11.5.2 with no temperature effect on final percentage germination or the intrinsic rate of germination. Model presented with mean and 95 % confidence intervals. $r^2 = 0.87$. n = 3.

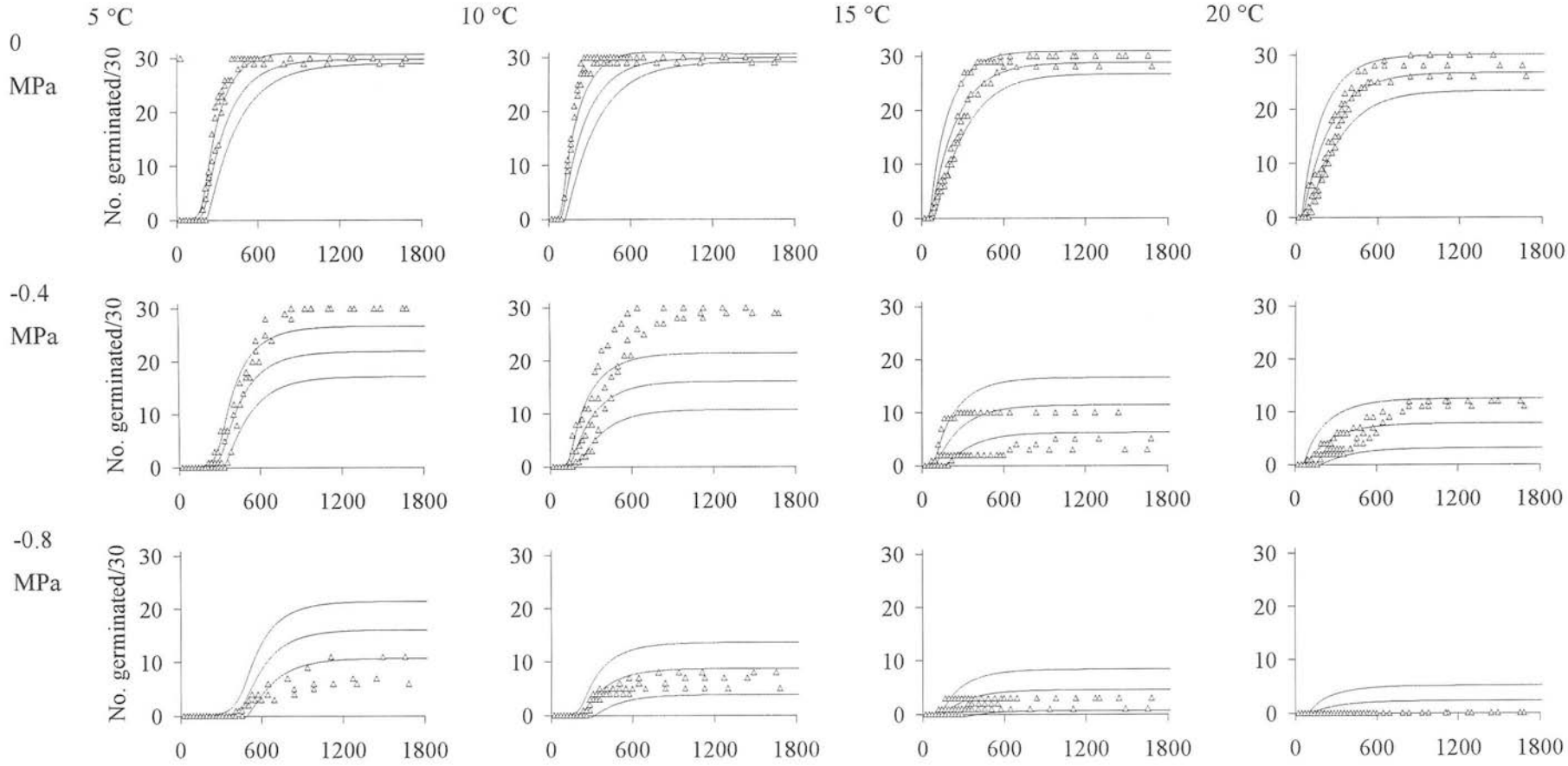


Figure 11.17: Fitted model compared with data for germination of the Northumberland population of *Galium aparine* at a range of constant temperatures and water potentials following exposure to red light. Model as defined in section 11.5.2 and presented with mean and 95 % confidence intervals. $r^2 = 0.88$.
n = 3.

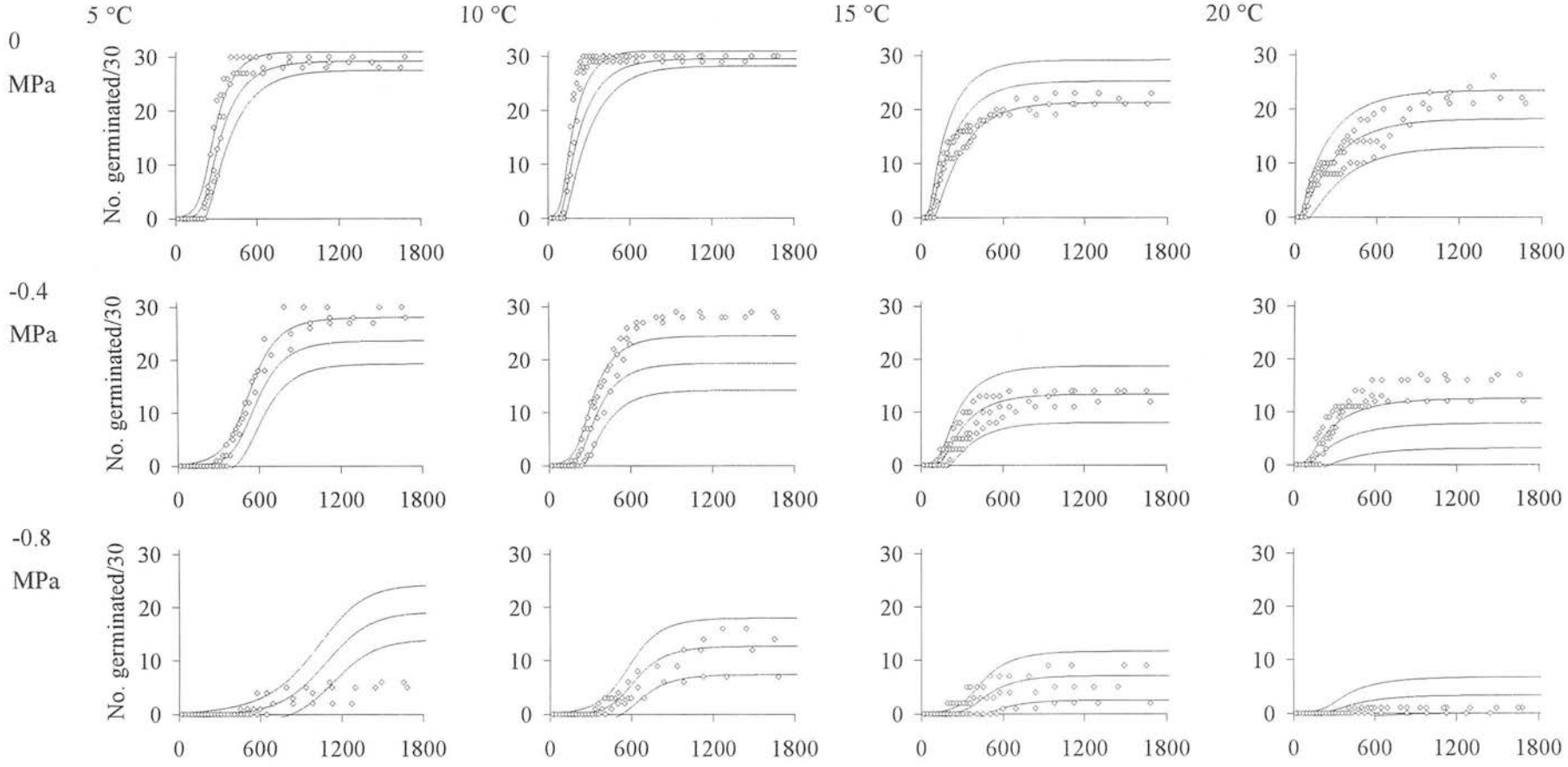


Figure 11.18: Fitted model compared with data for germination of the Northern Ireland of *Galium aparine* population at a range of constant temperatures and water potentials following exposure to red light. Model as defined in section 11.5.2 and presented with mean and 95 % confidence intervals. $r^2 = 0.89$.
n = 3.

11.7. *Discussion*

Table 11.16 summarises the notation used to describe the model as presented in the text and as shown in the Modelmaker (ver. 3.0.3) diagrams (see also Appendix 2). This is included to clarify parameter definitions and again it emphasises the different symbols used for thermal and hydrothermal time in this Chapter compared to the notation given in Chapters 6 & 7. This was to distinguish the different definitions of thermal and hydrothermal time used.

Table 11.16 also shows that the models developed were relatively simple. Dormancy was modelled by seasonal changes in soil temperature, whilst germination extent and timing were modelled by temperature and water potential. The model for seasonal changes in seed dormancy and identifying the proportion of germinable seeds required eight parameters and the model for germination timing required a further four parameters. This compared favourably with models for summer annual weed species presented by Vleeshouwers (1997), where a minimum of nine parameters were required to model seed dormancy and eight parameters were required to describe seed germination, solely with respect to temperature. Moreover it is clear that adoption of the stochastic formulation, in accounting for inherent variability in the processes and in allowing maximum likelihood estimation of parameters and their associated confidence intervals, gave the models presented in this chapter greater utility.

Variables	Modelmaker	Definition
t	t	Time
t_d	-	Time in the dormancy model
q_{td}	qtd	Proportion of 'non-dormant' seeds at time t in dormancy model
T	Temperature	Temperature (°C)
T_s	Ts	Soil temperature at 10 cm (°C)
ψ	WaterPotential	Water potential (MPa)
Tt		Thermal time: Cumulated temperature x time
HT	HT	Hydrothermal time: Thermal time multiplied by an exponential function of water potential

Estimated parameters	Modelmaker	Definition
q_0	q0	Proportion of 'non-dormant' seeds at $t = 0$
K_I	KI	Intrinsic rate of dormancy induction
K_R	KR	Intrinsic rate of dormancy release
β_{Ts}	BetaTs	Gradient of sigmoid function $s(T_s)$
μ_{Ts}	MuTs	Mean soil temperature for switch from dormancy release to dormancy induction
T_{opt}	Topt	Optimum temperature
σ_T	SigmaT	Defines the width of the optimum temperature
$\alpha_{\psi T}$	aPsiT	Controls the effect of temperature x water potential on germination extent
g_0	g0	Intrinsic rate of germination
a_ψ	aPsi	Controls the effect of water potential on hydrothermal time
β_{HT}	BetaHT	Gradient of sigmoid function $h(HT)$
μ_{HT}	MuHT	Mean hydrothermal time requirement

Table 11.16: Summary of variables and parameters used in seed dormancy and germination models. Note again that different symbols are used for thermal time and hydrothermal time compared to the notation given in Chapters 6 & 7. This is to emphasise that these represent different concepts of thermal time and hydrothermal time.

11.7.1. Models for seasonal dormancy and identification of the germinable seed fraction

Application of the models presented for dormancy and identification of the germinable seed fraction were undermined by insufficient data. For the *S. media* populations there was limited data to describe the rate of dormancy release and the effect of changing levels of dormancy on the range of temperatures at which exhumed seeds germinated. For *G. aparine*

there was sufficient data to describe the effect of changing levels of dormancy on the range of temperatures at which seeds germinated, but data were restricted to a single population and inadequate to describe changes in the rates of dormancy induction and release.

For *S. media*, limited data for dormancy release had two causes, firstly in relation to the late harvest of seeds prior to burial. This meant that initial seed dormancy was high (especially in the Perthshire population) and therefore data to estimate dormancy release was limited in the first autumn. Earlier harvest would likely have been associated with higher levels of initial seed dormancy (Baskin & Baskin, 1976; Baskin & Baskin, 1986). Secondly, the short time series of buried seed exhumations limited data availability in the second autumn. This was complicated by unexplained month to month variability in that second autumn. Consequently, for *S. media* parameters for rates of dormancy release and induction were poorly estimated, with wide confidence intervals for K_R and K_I. This could likely be resolved with data for more than one dormancy cycle.

Limited data to describe the effect of changing levels of dormancy on the range of temperatures at which exhumed seeds germinated was a consequence of limiting germination tests to just four test temperatures. A greater range of temperatures may have better illustrated more subtle consequences of seasonal changes in dormancy. However it is noteworthy that before July 1999, differences in germination extent according to temperatures within the range 5 to 20 °C were limited.

Overall the general pattern of seasonal changes in seed dormancy were similar for the different populations. The main differences were likely related to initial levels of seed dormancy (poor estimation of dormancy induction for the Perthshire and Caithness populations) and seed germination at 30 °C. For the Leicestershire population, seed germination throughout the year was limited at 30 °C and this was suggested to reflect selection pressure for reduced germination at high temperatures in a warmer/drier climate. This resulted in narrower σ_T, compared to the other populations.

For *G. aparine* there was a longer, but less frequent time series of seasonal changes in seed dormancy. This meant that dormancy release in the second autumn was recorded and fitted by the model. The data series also included germination tests of exhumed seeds at a greater range of temperatures and this allowed better assessment of the consequences of changing levels of dormancy for the range of temperatures at which exhumed seeds germinated (as

described by Vleeshouwers, Bouwmeester & Karssen, 1995). However infrequent recording make explain failure to adequately define rates of dormancy induction (K_I) and release (K_R). This could be improved by fitting the model to resampled data sets, and this remains to be tested. However the utility of this data set is further limited by restriction to consideration of a single replicate of a single population and it is unclear to what extent *G. aparine* populations vary in seasonal dormancy cycles. Although van der Weide (1993) presented additional, supporting data for seed lots buried at different times, it was not clear whether these seed populations represented different field populations.

11.7.2. Models for the timing of germination

The germination models presented gave better fits to the data than the hydrothermal time model described by Gummerson (1986) and evaluated in Chapters 6 & 7. On the simplest level this was evident in the r^2 statistics presented. More importantly the models presented in this Chapter were not restricted to consideration of only sub-optimal temperatures and for *G. aparine*, model fits were possible for all three populations. This, together with consideration of model formulation and systematic estimation of confidence intervals, meant that the models presented in this Chapter offer clear advantages over the hydrothermal models previously advocated. Moreover, given that the hydrothermal time model effectively only predicts timing of germination and not germination extent, this improvement has also been achieved without increasing the number of parameters required for the timing of germination component of the model (four parameters). Although it should be noted that much of the improved fit for these models is given because of the inclusion of additional the parameter q_0, to estimate initial seed dormancy. This is particularly important because weed seeds tend be more dormant and have more variable germination responses than crop plants, for which the hydrothermal time model was developed (Grundy, 1997). Following Vleeshouwers *et al.* (1995) where dormancy is defined as a continuous characteristic, with different levels of seed dormancy expressed according to test conditions, this initial dormancy is then modified by temperature and water potential to identify the germinable seed fraction. To date there has been limited consideration of how water potential interacts with seed dormancy itself and this requires further investigation.

Substantial variation between populations was observed for initial seed dormancy and subsequent response to temperature and water potential for both species. This meant that the

models were applied separately according to population and this variation was evident both in model parameters and in model predictions.

Regarding initial dormancy, it was clear for both *S. media* and *G. aparine*, that parameter estimates for $q0$ varied between populations, with no or limited overlap of confidence intervals.

For *S. media* and *G. aparine* populations, where preferred models included temperature effects on seed germination ($f(T)$), the optimum temperatures estimated by the model were similar for populations, tending to approximately 20 °C for *S. media* and 8 °C for *G. aparine*. This reflects observations that the temperature optimum for *G. aparine* is lower than that for *S. media*, and within the range 7 to 12 °C (summary of assorted authors in Malik & van den Born, 1988). For *S. media* reported optimum temperatures have varied between 10 and 20 °C (Roberts & Lockett, 1975; Grundy, 1997). This again suggests that *G. aparine* is better adapted to emerge at low temperatures and that emergence is more limited in warmer temperatures. This is supported by observations of reduced *G. aparine* emergence in warmer spring temperatures (Mortimer, 1990). For these same models, the parameter σ_T tended to be greater than 10 °C, with wide confidence intervals. This suggested that there was limited variation in germination extent and timing over a wide range of temperatures.

It was interesting to note the difference in optimum temperatures (T_{opt}) predicted by the dormancy and germination models. It is possible that this may represent an artefact of the extent of data used to fit the two models, with more data used in fitting the dormancy model. It may also reflect differences in behaviour related to maternal and/or storage effects as seeds were produced in different years (under different environmental conditions) and were either buried or dry stored.

Regarding the effect of the interaction between temperature and water potential on germination extent, there was limited variation between populations in the parameter $a_{\psi T}$. This suggests that this effect is relatively consistent for populations. Surprisingly, higher estimates were given for *G. aparine* and this did not reflect greater tolerance of reduced water potentials, particularly at lower temperatures.

For *S. media* there was also variation in the intrinsic rate of germination, and this was markedly faster for the Leicestershire population. Again this appeared contrary to

expectations as the start of germination tended to be slower in this population. However it should be remembered that the parameter g_0 represents the rate of germination after germination has started.

The onset of germination was determined by the hydrothermal time function. Hydrothermal time was calculated by multiplication of thermal time by an exponential function of water potential. The parameter a_ψ controlled the effect of water potential on hydrothermal time. For *S. media*, there were higher estimates of a_ψ for the Caithness population, representing greater delays in the onset of germination at low water potentials. For *G. aparine*, parameter values for a_ψ were higher than those recorded for *S. media* and differences between populations represented differences in the relative speed to the start of germination at reduced water potentials (fastest for the Northumberland population; slowest for the population from Northern Ireland). Interestingly for both species, the greater effects of reduced water potential on the onset of germination were recorded for the populations that produced the largest seeds and this may require further investigation. The seed size difference may also be reflected in differences in a_ψ between species.

This difference between species in speed to the start of germination was further defined by higher estimates of μ_{HT}, the mean hydrothermal time requirement, for *G. aparine*. These differences reflect patterns of emergence in the field, where *S. media* emergence precedes that of *G. aparine* (Mortimer, 1990). However delayed emergence for *G. aparine* tends to be offset by the production of larger, more competitive seedlings (Chapters 2 & 3). Between populations, differences in mean hydrothermal time requirements (μ_{HT}) also tended to reflect relative speed to emergence (for best model fits, lowest for Perthshire population of *S. media* and the Northumberland population of *G. aparine*).

The parameter β_{HT} represented the gradient of the hydrothermal time function and higher estimates tended to be associated with more synchronous germination, once hydrothermal time requirements were fulfilled. For *S. media* higher estimates of β_{HT} were given for the Caithness population and for *G. aparine* for the Northumberland population. Differences between species were not consistent.

It is evident that examination of the estimated parameters for the different populations and species gave insight into variation between populations and species in the germination process. The variation between populations was also evident in model predictions, where for

specific populations of both species, simplification of the basic model occurred without compromising model fits. For the Perthshire population of *S. media*, a simpler model adequately described germination, without temperature effects on germination extent and timing (r^2 = 0.83 compared to 0.85). Similar results, without temperature effects on germination extent and timing, were recorded for the North Yorkshire population of *G. aparine* (r^2 = 0.87 in both models). Simplification reduced the number of parameters in the germination model and this was possible because germination responses to test temperature for these populations were relatively flat.

Problems were encountered in fitting the Caithness model and these were associated with high levels of germination at 30 °C. Given that field exposure to 30 °C in Caithness was considered unlikely, this was resolved by restricting the model to consider germination between 5 and 20 °C. Removal of the temperature effect on germination extent and timing did not improve the model fit.

11.7.3. Further model development

The models presented have illustrated the difficulty of intraspecific variation in developing models of seed dormancy and germination for *S. media* and *G. aparine*. It is not clear how these differences can be resolved and further work is required to address this issue. This will likely involve attempts to generalise species behaviour and estimate associated confidence intervals.

The models presented have not been validated, i.e. they have not been used to predict new data. The particular need for additional data in the development of the dormancy models has already been stated. As noted, the r^2 statistics describe model goodness of fit and are likely biased estimates of the models predictive ability. Although these could be estimated with new data, cross validation methodology could also be utilised (section 4.3.5).

To date, application of the models has been limited to experimental data, with germination in constant temperature and water potential conditions. Further modelling and experimental effort is now required to test the consequences of variation in these conditions during seed germination.

Model application in varying environmental conditions is essential for model development for field application. Model development for field application will also require integration of additional factors that affect weed seed dormancy and germination, e.g. light, cultivation, maternal effects. However, model implementation in the commercial modelling package (Modelmaker ver. 3.0.3) presents a readily accessible and adaptable framework for further model development.

11.8. Conclusions

Simple models for *S. media* and *G. aparine* seed dormancy and germination have been developed and applied to existing data. Intraspecific variation between populations meant that separate models were fitted to different populations.

Dormancy was modelled by seasonal changes in soil temperature, whilst germination extent and timing were modelled by temperature and water potential. Eight parameters were required to model seasonal changes in seed dormancy and identify the proportion of germinable seeds and a further four parameters were required to model germination timing. The parameters used were readily interpretable and adoption of the stochastic formulation used, allowed maximum likelihood estimation of model parameters and their associated confidence intervals. Furthermore this stochastic model accounted for some of the observed variability between seeds.

Dormancy models were developed from similar ideas proposed for summer annual species by Vleeshouwers (1997). For *S. media* and *G. aparine*, the model predicted that dormancy was induced by late spring/early summer temperatures, and released in late summer. Complete dormancy was induced mid-summer in *S. media*, whilst for *G. aparine* actual germination was restricted to between 7 and 13 °C, although this was not adequately represented in the models presented. Further data is required to better describe dormancy release and induction in both species, intraspecific variation in seasonal dormancy changes for *G. aparine* and patterns of dormancy release in the second autumn for *S. media*.

Germination models gave markedly better fits to the data than the hydrothermal time model described by Gummerson (1986), and evaluated in Chapters 6 and 7. This was evident both in graphical representation of the fitted models compared to data and in the r^2 statistics presented. The models were also not restricted to consideration of only sub-optimal

temperatures and for *G. aparine*, model fits were presented for all three populations. Together with consideration of model formulation, this meant that the models presented offered clear advantages over hydrothermal time models (Gummerson, 1986) for modelling weed seed germination.

However the models illustrated the difficulty presented by intraspecific variation in developing models of seed dormancy and germination for *S. media* and *G. aparine*. It is not clear how these differences can be resolved and further work is required to address this issue.

Further work is also required to validate the models presented and to test seed germination in varying environmental conditions.

Future field application of these models relies on model application in varying environmental conditions. It will also involve model development to integrate additional factors that affect weed seed dormancy and germination. To support this, the modelling framework presented is readily adaptable.

Chapter 12. General discussion and summary

12.1. Intraspecific variation between populations

Substantial variation in seed and seedling characteristics exist between populations of *S. media* and *G. aparine* (Chapters 2 & 3). It has been inferred that this variation has a genetic basis as experiments used seeds harvested from plants that were grown for a generation in a common environment, in order to limit maternal effects. Although these 'genetic' differences were not directly assessed by molecular methods, characteristics of the selected populations were consistent (heritable) throughout the study.

For *S. media* populations, genetic differences (albeit interacting with conditions in the common environment) were responsible for nearly three-fold differences in seed size, ten-fold differences in the extent of seed germination and variation by a factor of two in seedling size at four leaves and seedling relative growth rate (Chapter 2). For *G. aparine* seed sizes varied by a factor of two, seed germination varied from 0 to 91 percent, seedling size at one whorl varied approximately two-fold and seedling relative growth rate varied by a factor of three (Chapter 3).

Analysis of the geographical basis of the variation between populations showed no general pattern, with neighbouring populations differing as much as widely dispersed populations in many cases. This was not wholly surprising given that many weed species are readily dispersed across the country as contaminants of crop seed and this wide-scale movement of weed seeds may limit the possibility of reproductive isolation and the development of local ecotypes (Cousens & Mortimer, 1995). However a number of smaller scale patterns were noted, including a trend to larger seed sizes for *S. media* populations from Scotland (Figure 2.5) and limited germination of *G. aparine* populations collected from environments dissimilar to that used to produce seeds in the common maternal environment (Table 3.3 & Table 3.4).

Among the selected *S. media* populations, the 'average' Leicestershire population was markedly more light sensitive, germination speed tended to be slower and germination was limited at 30 °C (Chapters 6, 8 & 10). It was suggested that these characteristics might reflect adaptations to limit seed germination from depth and at high temperatures, as

associated with limited water availability. It was suggested that these characteristics may have evolved under selection pressures associated with a relatively more intensively farmed landscape and/or moderate climate. Possible explanations for the characteristics of the Perthshire population were more problematic. The Perthshire population consistently flowered late and with relatively low reproductive effort, produced small seeds with low initial dormancy and rapid germination (Chapters 5, 6, 8 & 10). In contrast, the Caithness population consistently produced large seeds and exhibited unexpectedly high germination at 30 °C (Chapters 6, 8 & 10). It was suggested that for this population, from the far north of Scotland, this might reflect a lack of previous exposure to conditions of high temperature and drought, with associated selection pressures.

Among the selected *G. aparine* populations, differences between populations were less marked, but it should be noted that the contrasting populations were selected from a restricted sub-set of seeds included in the initial screening (Chapter 3). The North Yorkshire population had consistently higher levels of initial seed dormancy, compared to the populations from Northumberland and Northern Ireland, where initial seed dormancy was low (Chapters 7 & 9). Seeds produced by the Northern Ireland population were consistently larger than those produced by the other two populations (Chapters 3 & 9).

These results support hypothesis (1) that there are genetic (heritable) differences between populations of *S. media* and *G. aparine* in seed dormancy and germination characteristics.

12.2. Intraspecific variation within populations according to maternal effects

For both *S. media* and *G. aparine*, and according to population, substantial variation was evident in the characteristics of seeds produced following application of the herbicide, fluroxypyr at a range of reduced rates (Chapters 8 & 9). In common with Champion, Froud-Williams & Holland (1997, 1998) application of 1/2 dose fluroxypyr reduced subsequent seed size in two of the three *S. media* populations, and in one of the three *G. aparine* populations. In each of these cases this reduction in seed size was associated with reductions in seed germination at a range of temperatures. However for the other two populations of *G. aparine*, similar herbicide-related reductions in seed germination were also recorded, without reductions in seed size (Sections 9.4.2 & 9.4.3). For these two populations, effects of herbicide application on germination speed were also recorded. This suggests that herbicide-related maternal effects on seed germination cannot solely be explained by changes in seed

size distribution and may involve herbicide chemistry. Further variation would also likely be expected according to application timing and herbicide product (Shuma *et al.* 1995; Andersson, 1996). Maternal effects within populations were also shown in a trend to increased *S. media* seed size with additional nitrogen application in the maternal environment (Section 10.4.2).

These results support hypothesis (2) that differences in the maternal environment (in which seeds are produced) can account for significant variation in *S. media* and *G. aparine* germination characteristics within individual populations.

12.3. Intraspecific variation and the interaction between populations and maternal effects

As noted in 12.2, evidence of maternal effects following herbicide application was shown for several different populations. Comparison of these effects between populations showed that different populations of the same species had different responses to the same maternal effect, and as such these cannot be generalised (Chapters 8 & 9). This was further complicated by variation in response according to environmental test conditions. This was particularly evident among the *G. aparine* populations where for two of the populations, herbicide-related reductions in seed germination were only recorded for seeds tested at 20 °C (Figure 9.7).

These results support hypothesis (3) that populations interact with conditions in the maternal environment to produce further variation in *S. media* and *G. aparine* germination characteristics.

12.4. Implications of variation for modelling weed seed dormancy and germination

The extent of variation recorded and the complexity of interactions between genetic (heritable) population differences and maternal effects suggests that caution needs to be exercised in attempting to develop species models of weed seed dormancy and germination. Prior to consideration of modelling efforts, sections 12.5 and 12.6 review possible generalities in patterns of weed seed dormancy and germination and compare results for *S. media* and *G. aparine*.

12.5. Specific patterns of seasonal dormancy change

Seed dormancy for both *S. media* and *G. aparine* (van der Weide, 1993) varies seasonally. For populations of *S. media*, initial levels of seed dormancy varied markedly between populations, but subsequent seasonal changes in dormancy were generally consistent (Chapter 5). For both species, seed dormancy was induced in early summer. For *S. media* complete dormancy induction was recorded in mid-summer, whereas for *G. aparine* seed germination was reduced and recorded only between 7 and 13 °C (van der Weide, 1993). This may relate to differences in persistence in the soil seed bank and greater resilience of *G. aparine* to low water potentials (see section 12.6.2). For *G. aparine* dormancy relief in the autumn was clearly described (van der Weide, 1993), but for *S. media* dormancy relief was poorly described, largely because of insufficient data (Chapter 5).

12.6. Specific patterns of seed germination

12.6.1. Temperature

Temperature affects both the extent and timing of germination for *S. media* and *G. aparine*. For *S. media* germination extent tended to maximised at 20 °C, whereas for *G. aparine* this tended to 10 °C (Chapters 6 & 7). These optimal temperatures supported previous reports for *S. media* in Sobey (1981) and for *G. aparine* in Malik & van den Born (1988). The lower optimum temperature for *G. aparine* was also reflected in the T_{opt} parameter in the germination models (Chapter 11) and the hydrothermal time model predicted lower base temperatures for *G. aparine* compared with *S. media* (Chapters 6 & 7). For *S. media* germination occurred throughout the temperature range 5 to 30 °C, whereas for *G. aparine* germination was restricted to the range 5 to 20 °C, with marked reductions in germination extent at 20 °C. Germination speed was faster for *S. media* than *G. aparine*. For *S. media* germination speed increased as temperature increased from 5 to 20 °C whereas for *G. aparine* germination speed increased only between 5 and 10 °C. Germination speed at 5 °C was approximately 25 % faster for *S. media*, whereas at 20 °C this difference was 400 % (Chapters 6 & 7).

12.6.2. Water potential

Water potential also affects both the extent and timing of germination for *S. media* and *G. aparine* (Chapters 6 & 7). For both species, the extent of seed germination reduced with

decreasing water potential, except for *G. aparine* at 5 and 10 °C, with water potentials between 0 and –0.4 MPa. This suggested that germination of *G. aparine* was more tolerant of reduced water potential, albeit only at optimal temperatures. Van der Weide (1993) recorded similar tolerance of reduced water potential at optimal temperatures (8 compared to 14 °C). It is suggested that the difference between species may reflect differences in seed size, with larger seeds (and associated seed reserves) associated with greater seedling vigour (Stanton, 1984; Leishman, Westoby & Jurado, 1995).

For both species the effect of reduced water potential tended to be more marked at higher temperatures (≥ 30 °C for *S. media* (Figures 6.1, 6.2 & 6.3) and ≥ 15 °C for *G. aparine* (Figures 7.1, 7.2 & 7.3). This suggested selection pressure to limit germination in conditions of high temperatures and drought. For both species, the speed of germination decreased with reduced water potential (Tables 6.2 & 7.2). For all water potentials, germination speed for *S. media* remained faster than *G. aparine*. This again may reflect differences in seed and seedling size.

12.6.3. Light

Light affects the extent of germination in *S. media* and *G. aparine* (Grime *et al.*, 1981; Bliss & Smith, 1985). For *S. media* exposure to red light (photon flux density (PPFD): 3.84 ± 0.71 μmol m^{-2} s^{-1}) at imbibition increased the extent of germination, although there was marked variation between populations (Chapter 6). Germination also occurred where seeds were exposed to intermittent very low levels of green light, albeit to a lesser extent. Germination of *G. aparine* has been reported be inhibited by PPFDs greater than 70 μmol m^{-2} s^{-1} (Bliss & Smith, 1985) or 184 μmol m^{-2} s^{-1} (Grime *et al.*, 1981) and promoted compared to complete darkness, by PPFDs of 0.026 μmol m^{-2} s^{-1} (Bliss & Smith, 1985) or 4.5 μmol m^{-2} s^{-1} (Grime *et al.*, 1981). For *S. media* and *G. aparine* it therefore appears that exposure to low PPFD is effective at promoting seed germination, though both species are capable of some germination in the dark. For *S. media* there is evidence (Chapter 5) that this may interact with seed dormancy, such that seed burial may increase the proportion of seeds requiring light for germination. This supports similar observations by Wesson & Wareing (1969a, b)

12.6.4. Nitrogen

Nitrogen affects the extent of germination for both species and for *S. media* nitrogen was also shown to affect the timing of germination (Chapter 10). For *S. media*, nitrate ions have been shown to increase germination extent in the range 1 to 100 mmol l^{-1} and for *G. aparine* in the range 2 to 25 mmol l^{-1} (Ferris, 1988; van der Weide, 1993). For both species a poor relationship between germination extent and nitrate concentration have been demonstrated (Ferris, 1988, for *G. aparine*). For *S. media*, ammonium ions have also been shown to promote seed germination over the same range of concentrations, albeit to a lesser degree. This suggested a preference for nitrate ions in *S. media* seed germination (Figure 10.1). The effect of nitrogen on germination speed was illustrated with both nitrate and ammonium ions, with increased time to germination recorded at higher concentrations (100 mmol l^{-1}). However pot experiments recording *S. media* emergence in a range of soil nitrogen environments questions the significance of nitrogen in promoting seed germination in the field.

12.7. Modelling weed seed dormancy and germination

Chapter 11 presented models of seed dormancy and germination for *S. media* and *G. aparine*. In each case, model parameters were estimated separately for the different populations. This followed assessment of the extent of variation between populations.

The models presented were developed following a review of existing models (Chapter 4). Models were developed in a stochastic framework, which addressed statistical problems associated with least squares regression and allowed estimation of confidence intervals for both model output and model parameters by maximum likelihood methods (Hunter, Glasbey & Naylor, 1984). The timing and extent of germination in the soil seedbank was modelled in terms of the factors influencing dormancy (soil temperature) and germination (temperature and water potential). The dormancy model was developed from similar ideas to those proposed for summer annual species by Vleeshouwers (1997) and the germination model was developed independently following detailed examination and assessment of existing hydrothermal time models (Gummerson, 1986) (Chapters 6 & 7). The limitations of the hydrothermal time model are summarised below.

12.7.1. Assessment of existing hydrothermal time models

Chapter 6 examined application of the hydrothermal time model (Gummerson, 1986; Grundy, 1997) to germination time courses for *S. media* and Chapter 7 applied the same methodology to *G. aparine*. Although there was general appreciation of the concept of hydrothermal time, there were a number of problems associated with application and formulation of these models:

- Models were restricted to sub-optimal temperatures and this limited application to a sub-set of conditions, particularly for *G. aparine* where sub-optimal temperatures were defined as less than 10 °C (Figure 7.4).
- Models poorly predicted germination extent, particularly where there was marked seed dormancy.
- No confidence intervals were given for either the model output or parameter estimates.
- It was not clear how the model could be extended to incorporate the effects of other environmental factors, such as light or nitrogen.

These limitations, together with the general lack of consistency and adaptability in model formulation meant that an alternative framework for modelling weed seed germination was required. It should however be noted that in development of both the seed dormancy and timing of germination models described in sections 12.7.2 and 12.7.3, all the available data was used to fit the models and no independent model validation has been presented. As such the r^2 statistics given describe model goodness of fit.

12.7.2. Models for seasonal changes in seed dormancy and identification of the germinable fraction

The models developed represented seasonal variation (as described in section 12.5), according to soil temperature, in the proportion of germinable seeds at given test temperatures (Chapter 11). Eight parameters were required to model these seasonal changes, compared to fourteen used by Vleeshouwers (1997) to model seasonal dormancy changes for summer annual species. These fewer parameters have the additional advantage of being more readily interpretable. Goodness of fit statistics ranged from $r^2 = 0.76$ to 0.94 according to population for *S. media* and from $r^2 = 0.57$ to 0.58 according to fitted model for *G. aparine* (section 11.6).

For both species, rates of dormancy induction and relief were poorly estimated and this likely reflected insufficient time series data. For *S. media* data were restricted to a 12 month period, with substantial variation between replicates in the second autumn. Estimation of rates of dormancy relief and induction may also be improved by estimating the proportion of germinable seeds at a wider range of test temperatures. For *G. aparine*, the proportion of germinable seeds was estimated on five occasions over a 13 month period. However the proportion of germinable seeds was estimated over a wider range of temperatures and this allowed clearer assessment of the effect of dormancy on the range of temperatures at which seeds germinate (van der Weide, 1993). Incorporation of this dormancy effect in the *G. aparine* model produced a marginally improved fit, though it should be noted that these results were restricted to a single population.

12.7.3. Models for timing of germination

The germination models presented in Chapter 11 gave better fits to the data than the hydrothermal time model described by Gummerson (1986) and evaluated in Chapters 6 & 7. This was evident in the r^2 statistics and graphical representations (e.g. compare Figure 6.5 and Figure 11.9 for *S. media*; Figure 7.5 and Figure 11.17 for *G. aparine*). In comparison with the hydrothermal time models, the models developed were also not restricted to consideration of only sub-optimal temperatures and for *G. aparine*, model fits were possible for all three populations. Moreover, given that the hydrothermal time model effectively only predicts timing of germination and not germination extent, this improvement was achieved without increasing the number of parameters required for the timing of germination component of the model (four parameters). However it should be noted that much of the improved fit for these models is given because of the inclusion of additional the parameter q_0, to estimate initial seed dormancy. This was viewed as important because weed seeds tend be more dormant and have more variable germination responses than crop plants, for which the hydrothermal time model was developed (Grundy, 1997). Moreover the definition of the germinable fraction q_0, according to temperature and water potential supports the ideas of Vleeshouwers, Bouwmeester & Karssen (1995) in defining dormancy as a continuous characteristic, with different levels of seed dormancy expressed according to test conditions.

For both species, models were fitted separately to the different populations and the variation between populations was evident both in terms of estimated parameters and model predictions. Therefore the problem of integrating population behaviour into a general model

with potential field application remains problematic. This could require model refinement to reflect local population characteristics.

12.8. Future directions

The focus of future work is to validate and extend application of the models developed. Although population variability may limit potential field application, it is clear that adopting a modelling framework has proved useful in summarising understanding of seasonal changes in seed dormancy and germination response to environmental conditions for *S. media* and *G. aparine*.

Dormancy model extension would require further work to better describe seasonal patterns of *S. media* dormancy in the field and to assess variability between populations for dormancy in *G. aparine*. For *S. media* this would include a longer time series of change in the proportion of germinable seeds and germination tests at a greater range of temperatures to better assess changes in the width of temperature requirements for germination.

Germination model extension would require assessment of variability in germination responses to temperatures less than 5 °C and clearer quantification of the effect of light on seed germination (at a greater range of photon flux densities).

For both seed dormancy and germination, it is clear that understanding of maternal effects is limited, despite their potential significance for patterns of seed germination in the field. Further efforts are therefore required to assess the actual significance of maternal effects in field experiments and, as necessary, to develop the models to incorporate these effects. Efforts to explain maternal effects by observable changes in seed size and structure are useful, but such effects do not appear to be universal (Chapters 8, 9 & 10).

12.9. Summary

- There were genetic (heritable) differences between populations of *S. media* and *G. aparine* in seed dormancy and germination characteristics (as demonstrated by controlling maternal effects). These recorded differences were widespread, although no clear geographical patterns were identified.

- Differences in the maternal environment (in which seeds are produced) accounted for significant variability in *S. media* and *G. aparine* germination characteristics within a population (demonstrated by controlling for genetic (heritable) effects). For *S. media* herbicide application reduced subsequent seed size and germination in two of the three *S. media* populations. For *G. aparine* seed size was reduced in one population and both seed germination and germination speed were reduced in all three populations. Effects were dose-specific and differences in the maternal environment during seed production did not necessarily produce effects on subsequent seed germination. Maternal effects were not recorded for *S. media* seeds produced in a range of soil nitrogen environments, although seed size increased with higher levels of nitrogen.

- There were interactions between population and conditions in the maternal environment that produced further variability in *S. media* and *G. aparine* seed dormancy and germination characteristics. Different populations were affected to a different extent by differences in the maternal environment, as illustrated by the varying effect of herbicide application according to population.

- The extent of variation between populations meant that the quantitative models of seed dormancy and germination developed for *S. media* and *G. aparine* were fitted individually to the different populations. These models described the timing and extent of germination in the soil seedbank according to factors influencing dormancy (soil temperature) and germination (temperature and water potential).

- The extent of variation between populations was further emphasised by the need to modify model formulation to adequately represent seed dormancy and germination.

- Understanding of *S. media* and *G. aparine* seed responses to environmental conditions can be usefully synthesised by the development of these quantitative models and these models have effectively described the observed patterns of *S. media* and *G. aparine* seed dormancy and seed germination.

- Further work is now required to validate these models and establish the extent to which these models need to be adapted to account for additional environmental and management factors and for variation related to difference in maternal environments.

- The extent of intraspecific variation between populations and according to the maternal environment, will likely limit model development as a useful tool in predicting the extent of *S. media* and *G. aparine* seedling emergence in the field, although the goal of predicting the timing of emergence may be more attainable. However it is clear that

adoption of the modelling framework presented can prove a useful tool for both summarising current understanding and identifying future research needs.

References

Aarts, H.F.M. (1986). A computerized model for predicting changes in a population of *Galium aparine*. EWRS Symposium "Economic weed control". Stuttgart-Hohenheim: 277-284

Aastveit, A.H. & Martens H. (1986). ANOVA interactions interpreted by partial least squares regression. Biometrics 42: 829-844.

Alm, D.M., Stoller, E.W. & Wax, L.M. (1993). An index model for predicting seed germination and emergence rates. Weed Technology 7(3): 560-569.

Andersson, L. (1994a). Effects of MCPA and tribenuron methyl on seed production and seed size in annual weeds. Swedish Journal of Agrigultural Research 24: 49-56.

Andersson, L. (1994b). Seed production and seed weight of six weed species treated with MCPA. Swedish Journal of Agricultural Research 24: 95-100.

Andersson, L. (1995). The effects of dose and application timing on the seed production of three weed species treated with MCPA or tribenuron-methyl. Weed Research 35: 67-74.

Andersson, L. (1996). Characteristics of seeds and seedlings from weeds treated with sub-lethal herbicide doses. Weed Research 36: 55-64.

Andersson, L. & Milberg, P. (1998). Variation in seed dormancy among mother plants, populations and years of seed collection. Seed Science Research 8: 29-38.

Attwood, P.J. (Editor) (1985). Crop Protection Handbook – Cereals. British Crop Protection Council Publications: Croydon.

Bain, A.B. & Attridge, T.H. (1988). Shade –light mediated response in field and hedgerow populations of *Galium aparine* L. Journal of Experimental Botany 3(9): 1759-1764.

Barber, S.A. (1984). Soil nutrient bioavailability: A mechanistic approach. John Wiley & Sons Inc.: Toronto.

Baskin, C.C. & Baskin, J.M. (1988). Germination ecophysiology of herbaceous plant species in a temperate region. American Journal of Botany 75(2): 286-305..

Baskin, C.C. & Baskin, J.M. (1998). Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press: San Diego.

Baskin, J.M. & Baskin, C.C. (1976). High temperature requirement for after-ripening in seeds of winter annuals. New Phytologist 77: 619-624.

Baskin, J.M. & Baskin, C.C. (1979). Promotion of germination of *Stellaria media* seeds by light from a green safety lamp. New Phytologist 82: 381-383.

Baskin, J.M. & Baskin, C.C. (1986). Temperature requirements for after-ripening in seeds of nine winter annuals. Weed Research 26: 375-380.

Baskin, J.M. & Baskin, C.C. (1987). Temperature requirements for after-ripening in buried seeds of four summer annual weeds. Weed Research 27: 385-389.

Baskin, J.M. & Baskin, C.C. (1989a). Physiology of dormancy and germination in relation to seed bank ecology. In Leck, M.A., Parker, V.T. & Simpson, R.L. (Editors): Ecology of soil seed banks. Academic Press: San Diego: 53-66.

Baskin, J.M. & Baskin, C.C. (1989b). Role of temperature in regulating the timing of germination in soil seed reserves of *Thalspi arvense* L. Weed Research 29: 317-326.

Baskin, J.M. & Baskin, C.C. (1995). Variation in the annual dormancy cycle in buried seeds of the weedy winter annual, *Viola arvensis*. Weed Research 35: 353-362.

Baskin, J.M., Baskin, C.C. & Parr, J.C. (1986). Field emergence of *Lamium amplexicaule* L. and *L. purpureum* L. in relation to the annual seed dormancy cycle. Weed Research 26: 185-190.

Begon, M., Harper, J.L. & Townsend, C.R. (1990). Ecology – Individuals, populations and communities. 2nd Ed. Blackwell Scientific Publications: Oxford.

Benech-Arnold, R.L. & Sanchez, R.A. (1995). Modelling weed seed germination. In Kigel, J. & Galili, G. (Editors): Seed development and germination. Marcel Dekker Inc. New York: 545-566.

Benech-Arnold, R.L., Ghersa, C.M., Sanchez, R.A. & Insausti, P. (1990). A mathematical model to predict *Sorghum halapense* (L.) Pers. seedling emergence in relation to soil temperature. Weed Research 30: 91-99.

Benjamin, L.R. (1990). Variation in time of seedling emergence within populations: a feature that determines individual growth and development. Advances in Agronomy 44: 1-25.

Bewley, J.R. & Black, M. (1994). Seeds: Physiology of development and germination. 2nd Ed. Plenum Press: New York.

Bliss, D. & Smith, H. (1985). Penetration of light into soil and its role in the control of seed germination. Plant, Cell & Environment 8: 475-483.

Boutsalis, P. & Powles, S.B. (1998). Seedbank characteristics of herbicide resistant and susceptible *Sisymbrium orientale*. Weed Research 38: 389-395.

Bouwmeester, H.J. & Karssen, C.M. (1989). Environmental factors influencing the expression of dormancy patterns in weed seeds. Annals of Botany 63: 113-120.

Bouwmeester, H.J. & Karssen, C.M. (1992). The dual role of temperature in the regulation of the seasonal changes in dormancy and germination of seeds of *Polygonum persicaria* L. Oecologia 90: 88-94.

Bouwmeester, H.J. & Karssen, C.M. (1993a). Seasonal periodicity in germination of seeds of *Chenopodium album*. Annals of Botany 72: 463-473.

Bouwmeester, H.J. & Karssen, C.M. (1993b). Annual changes in dormancy and germination of seeds of *Sisymbrium officinale* (L.) Scop. New Phytologist 124: 179-191.

Bouwmeester, H.J. & Karssen, C.M. (1993c). The effect of environmental conditions on the annual dormancy pattern of seeds of *Spergula arvensis*. Canadian Journal of Botany 71: 64-73.

Bradford, K.J. (1995). Water relations in seed germination. In Kigel, J. & Galili, G. (Editors): Seed development and germination. Marcel Dekker Inc. New York: 351-396.

Brenchley, W.E. & Warington, K. (1930). The weed seed population of an arable soil. I. Numerical estimation of viable seeds and observations on their natural dormancy. Journal of Ecology 18: 235-272.

Brenchley, W.E. & Warington, K. (1933). The weed seed population of arable soil. II. Influence of crop, soil, and methods of cultivation upon the relative abundance of viable seeds. Journal of Ecology 21: 103-127.

Brown, R.F. & Mayer, D.G. (1988). Representing cumulative germination: 2. The use of the Weibull function and other empirically derived functions. Annals of Botany 61: 127-138.

Champion, G.T., Froud-Williams, R.J. & Holland, J.M. (1997). The implications of reduced doses of fluroxypyr and wheat cultivar choice on the seed production, growth

and development of the progeny of common field speedwell. Brighton Crop Protection Conference – Weeds: 111-116.

Champion, G.T., Froud-Williams, R.J. & Holland, J.M. (1998). The effect of reduced rates of fluroxypyr on the seed size and germination of common field speedwell, *Veronica persica*. Aspects of Applied Biolgy 51. Weed seedbanks: determination, dynamics and manipulation. AAB: Wellesbourne: 143-146.

Chancellor, R.J. (1986). Decline of arable weed seeds during 20 years under grass and the periodicity of seedling emergence after cultivation. Journal of Applied Ecology 23: 631-637.

Chancellor, R.J. & Peters, N.C.B. (1972). Germination periodicity, plant survival and seed production in populations of *Avena fatua* L. growing in spring barley. Proceedings of the 11th British Weed Control Conference. Brighton: 218-225.

Cook, R. (1980). The biology of seeds in the soil. In Solbrig, O.T. (Editor): Demography and evolution in plant populations. Blackwell Scientific Publications, Oxford: 107-130.

Cook, S.K. & Clarke, J.H. (1997). The effect of six years of reduced rate herbicide use and rotation on weed levels, yields and profitability – TALISMAN results. Brighton Crop Protection Conference - Weeds: 705-710.

Cook, S.K., Hill, P.E. & Green M. (1996). The effects of a low-input regime on weed populations in contrasting rotations of the TALISMAN experiment. Aspects of Applied Biology 47: 230-236.

Copeland, L.O. & McDonald, M.B. (1995). Principles of seed science and technology. 3rd Ed. Chapman & Hall: New York.

Courtney, A.D. (1968). Seed dormancy and field emergence in *Polygonum aviculare*. Journal of Applied Ecology 5: 675-684.

Cousens, R. & Mortimer M. (1995). Dynamics of weed populations. Cambridge University Press: Cambridge.

Crawford, T.J. & Jones, D.A. (1977). Variation in colour of keel petals in *Lotus corniculatus* L. I. The polymorphism in Western Europe. Heredity 39: 313-325.

Dahal, P. & Bradford, K.J. (1990). Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. II. Germination at sub-optimal temperatures. Journal of Experimental Botany 41: 1441-1453.

Dahal, P., Bradford, K.J. & Jones R.A. (1990). Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. I. Germination at reduced water potential. Journal of Experimental Botany 41: 1431-1439.

Derkx, M.P.M. & Karssen, C.M. (1993). Changing sensitivites to light and nitrate but not to gibberellins regulates seasonal dormancy patterns in *Sisymbrium officinale* seeds. Plant, Cell and Environment 16: 469-479.

Efron, B. & Tibishirani, R.J. (1993). An introduction to the bootstrap. Chapman & Hall: New York.

Egley, G.H. (1995). Seed germination in soil: dormancy cycles. In Kigel, J. & Galili, G. (Editors): Seed development and germination. Marcel Dekker Inc.: New York: 529-544.

Egli, D.B., Te Krony, D.M. & Wiralaga, R.A. (1990). Effect of soybean vigour and seed size on seedling growth. Journal of Seed Technology 14: 1-2.

Ellis, R.H., Covell, S., Roberts, E.H. & Summerfield, R.J. (1986). The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. Journal of Experimental Botany 37 (183): 1503-1515.

Ellis, R.H., Hong, T.D. & Roberts E.H. (Editors) (1985). Handbook of seed technology for genebanks: Volume I – Principles and methodology. International Board for Plant Genetic Resources: Rome.

Ellis, R.H., Simon, G. & Covell, S. (1987). The influence of temperature on seed germination rate in grain legumes. III. A comparison of five faba bean genotypes at constant temperatures using a new screening method. Journal of Experimental Botany 38 (191): 1033-1043.

Environmental Protection Agency (1998). http://www.epa.gov/opprd001/factsheets/fluroxyp.htm

Fawcett, R.S. & Slife, F.W. (1978a). Effects of 2,4-D and dalapon on weed seed production and dormancy. Weed Science 26(6): 543-547.

Fawcett, R.S. & Slife, F.W. (1978b). Effects of field application of nitrate on weed seed germination and dormancy. Weed Science 26(6): 594-596.

Fenner, M. (1995). Ecology of seedbanks. In Kigel, J. & Galili, G. (Editors): Seed development and germination. Marcel Dekker Inc.: New York: 507-528.

Fernandez Quintanilla, C. (1988). Studying the population dynamics of weeds. Weed Research 28: 443-447.

Ferris, R. (1988). Inter-population variation in *Galium aparine* L. (cleavers). PhD Thesis: University of Reading.

Finch-Savage, W.E. & Phelps, K. (1993). Onion (*Allium cepa* L.) seedling emergence patterns can be explained by the influence of soil temperature and water potential on seed germination. Journal of Experimental Botany 44 (259): 407-414.

Finney, D.J. (1971). Probit analysis. 3rd Edition. Cambridge University Press: Cambridge.

Fisher, N.M., Davies, D.H.K. & Whytock, G.P. (1993). Reliability of broad-leaved weed control in cereals using low doses of herbicides. Brighton Crop Protection Conference – Weeds: 1223-1228.

Fitter, A.H. & Peat, H.J. (1994). The Ecological Flora Database. Journal of Ecology 82: 415-425.

Forcella, F. (1992). Prediction of weed seedling densities from buried seed reserves. Weed Research 32: 29-38.

Freckleton, R.P. & Watkinson, A.R. (1998). Predicting the determinants of weed abundance: a model for the population dynamics of *Chenopodium album* in sugar beet. Journal of Applied Ecology 35: 904-920.

Froud-Williams, R.J. (1985). The biology of cleavers (*Galium aparine*). Aspects of Applied Biology 9: 189-195.

Froud-Williams, R.J. & Ferris-Kaan, R. (1991). Intraspecific variation among populations of cleavers (*Galium aparine* L.). Brighton Crop Protection Conference - Weeds: 1007-1014.

Froud-Williams, R.J., Chancellor, R.J. & Drennan, D.S.H. (1983). Influence of cultivation regime upon buried weed seeds in arable cropping systems. Journal of Applied Ecology 20: 199-208.

Froud-Williams, R.J., Drennan, D.S.H. & Chancellor, R.J. (1984). The influence of burial and dry storage upon cyclic changes in dormancy, germination and response to light in seeds of various arable weeds. New Phytologist 96: 473-481.

Gange, A.C., Brown, V.K. & Farmer, L.M. (1992). Effects of pesticides on the germination of weed seeds: implications for manipulative experiments. Journal of Applied Ecology 29: 303-310.

Garcia-Huidbro, J., Monteith, J.L. & Squire, G.R. (1982). Time, temperature and germination of pearl millet (*Pennisetum typhoides* S & H). I. Constant temperature. Journal of Experimental Botany 33: 288-296.

Genstat 5 (1999). http://www.res.bbsrc.ac.uk/stats/ Distributed by NAG http://www.nag.com/stats/TT.html

Ghersa, C.M. & Holt, J.S. (1995). Using phenology prediction in weed management: a review. Weed Research 35: 461-470.

Gibson, G.J., Gilligan, C.A. & Kleczkowski, A. (1999). Predicting variability in biological control of a plant-pathogen system using stochastic models. Proceedings of the Royal Society of London Series B: Biological Sciences 266(1430): 1743-1753.

Goudey, J.S., Saini, H.S. & Spencer, M.S. (1986). Seed germination of wild mustard (*Sinapis arvensis*): factors required to break primary dormancy. Canadian Journal of Botany 65: 849-852.

Goudey, J.S., Saini, H.S. & Spencer, M.S. (1988). Role of nitrate in regulating germination of *Sinapis arvensis* L. (wild mustard). Plant, Cell and Environment 11: 9-12.

Grime, J.P. (1989). Seed banks in ecological perspective. In Leck, M.A., Parker, V.T. & Simpson, R.L. (Editors): Ecology of soil seed banks. Academic press: San Diego: xv-xxi.

Grime, J.P. & Hunt, R. (1975). Relative growth rate: its range and adaptive significance in a local flora. Journal of Ecology 63: 393-422.

Grime, J.P., Hodgson J.G. & Hunt, R. (1988). Comparative plant ecology: a functional approach to common plant species. Unwin Hyman: London.

Grime, J.P., Mason, G., Curtis, A.V., Rodman, J., Band, S.R., Mowforth, M.A.G., Neal, A.M. & Shaw, S. (1981). A comparative study of germination characteristics in a local flora. Journal of Ecology 69: 1017-1059.

Grundy, A.C. (1997). The influence of temperature and water potential on the germination of seven different dry stored seed lots of *Stellaria media*. Weed Research 37257-266.

Grundy, A.C., Froud-Williams, R.J. & Boatman, N.D. (1995). Maternal effects in progeny of field pansy (*Viola arvensis*) subjected to different herbicide and nitrogen rates. Annals of Applied Biology 127: 343-352.

Grundy, A.C., Mead, A. & Burston, S. (1999). Modelling the effect of cultivation on seed movement with application to the prediction of weed seedling emergence. Journal of Applied Ecology 36: 663-678.

Gummerson, R.J. (1986). The effect of constant temperature and osmotic potentials on the germination of sugar beet. Journal of Experimental Botany 37 (179): 729-741.

Gutterman, Y. (1980). Influences on seed germinability: phenotypic maternal effect during seed maturation. Israel Journal of Botany 29 (1-2): 105-107.

Gutterman, Y. (1992). Maternal effect on seeds during development. In Fenner, M. (Editor): Seeds - The ecology of regeneration in plant communities. CAB International: Wallingford: 27-59.

Hald, A.B. (1993). Seed setting of a non-target wild plant species at sublethal herbicide doses. 8th EWRS Symposium "Quantitative approaches in weed and herbicide research and their practical application". Braunschweig: 631-638.

Hall, D.O., Scurlock, J.M.O., Bolhar-Nordenkampf, H.R., Leegood, R.C. & Long, S.P. (Editors) (1993). Photosynthesis and production in a changing environment - a field and laboratory manual. Chapman & Hall: London.

Hall, J.C. & Romano, M.L. (1995). Morphological and physiological differences between the auxinic herbicide-susceptible (S) and -resistant (R) wild mustard (*Sinapis arvensis* L.) biotypes. Pesticide Biochemistry and Physiology 52: 149-155.

Harper, J.L. (1977). Population biology of plants. Academic Press: London.

Harper, J.L. & Ogden, J. (1970). The reproductive strategy of higher plants. 1. The concept of strategy with special reference to *Senecio vulgaris*. Journal of Ecology 58: 681-698.

Harvey, P.H. & Forcella, F. (1993). Vernal seedling emergence model for common lambsquarters (*Chenopodium album*). Weed Science 41: 309-316.

Harvey, P.H., Read, A.F. & Nee, S. (1995a). Why ecologists need to be phylogenetically challenged. Journal of Ecology 83: 535-536.

Harvey, P.H., Read, A.F. & Nee, S. (1995b). Further remarks on the role of phylogeny in comparative ecology. Journal of Ecology 83: 733-734.

Haynes, R.J. (1986) Mineral nutrition in the plant-soil system. Academic Press: Orlando.

Hegarty, T.W. (1973). Temperature coefficient (Q10), seed germination and other biological processes. Nature 243: 305-306.

Hendry, G.A.F. & Grime, J.P. (Editors) (1993). Methods in comparative plant ecology: a laboratory manual. Chapman & Hall: London.

Hill, A.L., Courtney, A.D. & Harvey, B.M.R. (1996). An assessment of the possible reasons for differential tolerance to fluroxypyr in selected populations of *Galium aparine*. Weed Research 36: 15-20.

Hodgson, J.G. & Mackey, J.M.L. (1986). The ecological specialisation of dicotyledonous families within a location: Some factors constraining optimisation of seed size. New Phytologist 104: 497-515.

Holm, L., Doll, J., Holm, E., Pancho, J. & Herberger, J. (Editors) (1997). World weeds - natural histories and distribution. J. Wiley & Sons Inc.: New York.

Hume, L. & Shirriff, S. (1989). The effect of 2,4-D on growth and germination of Lamb's quarters (*Chenopodium album* L.) plants having different degrees of tolerance. Canadian Journal of Plant Science 69: 897-902.

Hunt, R. (1978a). Plant growth analysis. Edward Arnold: London.

Hunt, R. (1978b). Demography versus plant growth analysis. New Phytologist 80: 269-272.

Hunter, E.A., Glasbey, C.A. & Naylor, R.E.L. (1984). The analysis of data from germination tests. Journal of Agricultural Science 102: 207-213.

Jones, H.G. (1992). Plants and microclimate. 2nd Ed. Cambridge University Press: Cambridge.

Jornsgard, B., Rasmussen, K, Hill, J. & Christiansen, J.L. (1996). Influence of nitrogen on competition between cereals and their natural weed populations. Weed Research 36: 461-470.

Kane, M. & Cavers, P.B. (1992). Patterns of seed weight distribution and germination with time in a weedy biotype of proso millet (*Panicum milaceum*). Canadian Journal of Botany 70 (3): 562-567.

Karssen, C.M. & De Vries, B. (1983). Regulation of dormancy and germination by nitrogenous compounds in the seeds of *Sisymbrium officinale* L. (hedge mustard). Influence of environmental factors on herbicide performance and crop and weed biology. Aspects of Applied Biology 4: 47-54.

Karssen, C.M. & Hilhorst, H.W.M. (1992). Effect of the chemical environment on seed germination. In Fenner, M. (Editor): Seeds - The ecology of regeneration in plant communities. CAB International: Wallingford: 327-348.

Kigel, J. & Galili, G. (Editors) (1995). Seed development and germination. Marcel Dekker Inc.: New York.

Krebs, C.J. (1972). Ecology: The experimental analysis of distribution and abundance. Harper & Row: New York.

Kremer, E. & Lotz, L.A.P. (1998a). Germination and emergence charactersitics of triazine-susceptible and triazine-resistant biotypes of *Solanum nigrum*. Journal of Applied Ecology 35: 302-310.

Kremer, E. & Lotz, L.A.P. (1998b). Emergence depth of triazine susceptible and resistant *Solanum nigrum* seeds. Annals of Applied Biology 132: 277-288.

Kropff, M.J., Spitters, C.J.T., Schnieders, B.J., Joenje, W. & De Groot, W. (1992). An eco-physiological model for interspecific competition, applied to the influence of *Chenopodium album* L. on sugar beet. II Model evaluation. Weed Research 32: 451-463.

Kryger Jensen, P. (1995). Effect of light environment during soil disturbance on germination and emergence pattern of weeds. Annals of Applied Biology 127: 561-571.

Kudsk, P. (1989). Experience with reduced herbicide doses in Denmark and the development of the concept of factor-adjusted doses. Brighton Crop Protection Conference - Weeds: 545-554.

Kudsk, P., Mathiassen, S.K. & Cotterman, J.C. (1995). Sulfonylurea resistance in *Stellaria media* [L.] Vill. Weed Research 35: 19-24.

Law, R. (1983). A model for the dynamics of a plant population containing individuals classified by age and size. Ecology 64: 224-230.

Lawson, H.M. & Wiseman, J.S. (1979). Competition between weeds and transplanted spring cabbage: Effects of nitrogen top dressing. Horticultural Reseach 19: 25-34.

Leishman, M.R., Westoby, M. & Jurado, E. (1995). Correlates of seed size variation: a comparison among five temperate floras. Journal of Ecology 83: 517-530.

Ligneau, L.A.M. & Watt, T.A. (1995). The effects of domestic compost upon the germination and emergence of barley and six arable weeds. Annals of Applied Biology 126: 153-162.

Malik, N. & van den Born, W.H. (1987). Germination response of *Galium spurium* L. to light. Weed Research 27: 251-258.

Malik, N. & van den Born, W.H. (1988). The biology of Canadian weeds 86: *Galium aparine* L. and *Galium spurium* L. Canadian Journal of Plant Science 68: 481-499.

Mallik, M.A.B., Puchala, R. & Grosz, F.A. (1994). A growth inhibitory factor from lambsquarters (*Chenopodium album*). Journal of Chemical Ecology 20 (4): 957-967.

Martinez-Ghersa, M.A., Satorre, E.H. & Ghersa, C.M. (1997). Effect of soil water content and temperature on dormancy breaking and germination in three weeds. Weed Science 45: 791-797.

Mayer, A.M. & Poljakoff-Mayer, A. (1975). The germination of seeds. MacMillan: New York.

McGinley, M.A., Temme, D.H. & Geber, M.A. (1987). Parental investment in offspring in variable environments: theoretical and empirical considerations. American Naturalist 130 (3): 370-398.

Mead, A., Grundy, A.C. & Bond, W. (1996). Modelling the effect of weed-seed distribution in the soil profile on seedling emergence. Weed Research 36: 375-384.

Michel, B.E. (1983). The evaluation of water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiology 72: 739-742.

Milberg, P. (1997). Weed seed germination after short term light exposure: germination rate, photon fluence response and interaction with nitrate. Weed Research 37: 157-164.

Milberg, P. & Andersson, L. (1997). Seasonal variation in dormancy and light sensitivity in buried seeds of eight annual weed species. Canadian Journal of Botany 75: 1998-2004.

Milberg, P. & Andersson, L. (1998). Dormancy dynamics in buried weed seeds. Aspects of Applied Biology 51. Weed seedbanks: determination, dynamics and manipulation. AAB: Wellesbourne: 153-158.

Mitchelson, K.R., Knox, O., Cheng, J., Ford, M.A., Wilson, F. & Atkinson, D. (1995). Molecular markers for genetic diversity in cleavers (*Galium aparine*). Brighton Crop Protection Conference - Weeds: 451-458.

Mortimer, A.M. (1987). The population ecology of weeds – implications for integrated weed management, forecasting and conservation. Brighton Crop Protection Conference - Weeds: 935-944.

Mortimer, A.M. (1990). The biology of weeds. In Hance, R.J. & Holly, K. (Editors): Weed Control Handbook. 8th Ed. Blackwell Scientific: Oxford: 1-42.

Murdoch, A.J. & Ellis, R.H. (1992). Longevity, viability and dormancy. In Fenner, M. (Editor) Seeds: The ecology of regeneration in plant communities. CAB International: Wallingford.

Murdoch, A.J., Roberts, E.H. & Goedert, C.O. (1989). A model for germination responses to alternating temperatures. Annals of Botany 63: 97-111.

Numerical Algorithms Group (1999) http://www.nag.co.uk

Ogilvy, S.E., Turley, D.B., Cook, S.K., Fisher, N.M., Holland, J, Prew, R.D. & Spink, J. (1995). LINK integrated farming systems: A considered approach to crop protection. In McKinlay R.G. & Atkinson, D.A. (Editors) Integrated cop protection: Towards sustainability? BCPC Symposium no. 63: 331-338.

Panetta, F.D. & Randall, R.P. (1993). Variation between *Emex australis* populations in seed dormancy/non-dormancy cycles. Australian Journal of Ecology 18: 275-280.

Pons, T.L. (1992). Seed response to light. In Fenner, M. (Editor): Seeds - the ecology of regeneration. CAB International: Wallingford: 259-284.

Probert, R.J. (1992). The role of temperature in germination ecophysiology. In Fenner, M. (Editor): Seeds - the ecology of regeneration. CAB International: Wallingford: 285-326.

Quinn, J.A. & Colosi, J.C. (1977). Separating genotype from environment in germination ecology experiments. American Midland Naturalist 97: 484-489.

Rasmussen, I.A. (1993). Seed production in *Chenopodium album* in spring barley sprayed with different herbicides at normal to very low doses. 8th EWRS Symposium 'Quantitative approaches to weed and herbicide research and their practical application.' Braunschweig: 639-646.

Reader, R.J. (1993). Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. Journal of Ecology 81: 169-175.

Rees, M. (1997). Seed dormancy. In Crawley, M.J. (Editor): Plant ecology. 2nd Ed. Blackwell Science: Oxford: 214-238.

Renshaw, E. (1991). Modelling biological populations in space and time. Cambridge University Press: Cambridge.

Riley, D. & Eagle, D. (1990). Herbicides in soil and water. In Hance, R.J. & Holly, K. (Editors) Weed control handbook – Principles. Blackwell Scientific Publishers: Oxford.

Roach, D.A. & Wulff, R.D. (1987). Maternal effects in plants. Annual Review of Ecology and Systematics 18: 209-235.

Roberts, E.H. & Benjamin, S.K. (1979). The interaction of light, nitrate and alternating temperatures on the germination of *Chenopodium album, Capsella bursa-pastoris* and *Poa annua* before and after chilling. Seed Science and Technology 7: 379-392.

Roberts, E.H. & Smith, R.D. (1977). Dormancy and the pentose phosphate pathway. In Khan, A.A. (Editor) The physiology and biochemistry of seed dormancy and germination. Elsevier Biomedical Press: Amsterdam.

Roberts, H.A. (1962). Studies on the weeds of vegetable crops. II. Effect of six years of cropping on the weed seeds in the soil. Journal of Ecology 46: 759-768.

Roberts, H.A. (1964). Emergence and longevity in cultivated soil of seeds of some annual weeds. Weed Research 4: 296-307.

Roberts, H.A. & Boddrell, J.E. (1983). Seed survival and periodicity of seedling emergence in eight species of Cruciferae. Annals of Applied Biology 103: 301-304.

Roberts, H.A. & Feast, P.M. (1973). Emergence and longevity of seeds of annual weeds in cultivated and uncultivated soil. Journal of Applied Ecology 10: 133-143.

Roberts, H.A. & Lockett, P.M. (1975). Germination of buried and dry-stored seeds of *Stellaria media*. Weed Research 15: 199-204.

Roberts, H.A. & Neilson, J.E. (1980). Seed survival and periodicity of seedling emergence in some species of *Atriplex, Chenopodium, Polygonum* and *Rumex*. Annals of Applied Biology 94: 111-120.

Roberts, H.A. & Potter, M.E. (1980). Emergence patterns of weed seedlings in relation to cultivation and rainfall. Weed Research 20: 377-386.

Roberts. H.A. (1984). Crop and weed patterns in relation to time of cultivation and rainfall. Annals of Applied Biology 105: 263-275.

Rundel, P.W. & Jarrell, W.M. (1989). Water in the environment. In Pearcy, R.W., Ehleringer, J., Mooney, H.A. & Rundel, P.W. (Editors). Plant physiological ecology – Field methods and instrumentation. Chapman & Hall: London.

Saini, H.S., Bassi, P.K. & Spencer M.S. (1985a). Seed germination in *Chenopodium album* L.: Relationships between nitrate and effects of plant hormones. Plant Physiology 77: 940-945.

Saini, H.S., Bassi, P.K. & Spencer M.S. (1985b). Seed germination in *Chenopodium album* L.: Further evidence for the dependence of growth regulators on nitrate availability. Plant, Cell & Environment 8: 707-711.

Sarukhan, J. & Gadgil, M. (1974). Studies on plant demography:*Ranunculus repens* L., *R. bulbosus* L. and *R. acris L.* III. A mathematical model incorporating multiple modes of reproduction. Journal of Ecology 62: 921-936.

Schimpf, D.J. & Palmblad, I.G. (1980). Germination repsonse of weed seeds to soil nitrate and ammonium with and without simulated overwintering. Weed Science 28 (2): 190-193.

Scopel, A.L., Ballare, C.L. & Radosevich, S.R. (1994). Photostimulation of seed germination during soil tillage. New Phytologist 126: 145-152.

Selman, M. (1970). The population dynamics of *Avena fatua* (wild oats) in continuous spring barley: Desirable frequency of spraying with tri-allate. Proceedings of 10th British Weed Control Conference: 1176-1188.

Shuma, J.M., Quick, W.A., Raju, M.V.S. & Hsiao, A.I. (1995). Germination of seeds from plants of *Avena fatua* treated with glyphosate. Weed Research 35: 249-255.

Simpson, R.L., Leck, M.A. & Parker, V.T. (1989). Seed banks: general concepts and methodological issues. In Leck, M.A., Parker, V.T. & Simpson, R.L. (Editors): Ecology of soil seed banks. Academic Press: San Diego: 3-7.

Sobey, D.G. (1981). Biological flora of the British Isles No. 150: *Stellaria media* (L.) Vill. Journal of Ecology 69: 311-335.

Spitters, C.J.T. (1989). Weeds: population dynamics, germination and competition. In Rabbinge, R., Ward, S.A. & van Laar, H.H. (Editors): Simulation and systems management in crop protection. Pudoc: Wageningen: 182-216.

Stanton, M. (1984). Seed size variation in wild radish: effect of seed size on components of seedling and adult fitness. Ecology 65 (4): 1105-1112.

Tait, J. & Pitkin, P.H. (1995). New technology for environmental benefits: Opportunities for industry. Brighton Crop Protection Conference – Weeds: 593-602,

Thompson, K. & Grime, J.P. (1979). Seasonal variation in the seedbanks of herbaceous species in 10 contrasting habitats. Journal of Ecology 67: 893-921.

Thompson, K. & Grime, J.P. (1983). A comparative study of germination responses to diurnally-fluctuating temperatures. Journal of Applied Ecology 20: 141-156.

Thompson, K. & Whatley, J.C. (1983). Germination responses of naturally-buried weed seeds to diurnal temperature fluctuations. Aspects of Applied Biology 4: 71-77.

Thompson, K. & Whatley, J.C. (1984). A thermogradient bar apparatus for the study of germination requirements of buried seeds *in situ*. New Phytologist 96: 459-471.

Thompson, K., Bakker, J.P. & Bekker, R.M. (1997). The soil seedbanks of North West Europe: Methodology, density & longevity. Cambridge University Press: Cambridge.

Thompson, K., Band, S.R. & Hodgson, J.G. (1993). Seed size and shape predict persistence in soil. Functional Ecology 7: 236-241.

Thompson, K., Grime, J.P. & Mason, G. (1977). Seed germination in response to diurnal fluctuations of temperature. Nature 267: 147-149.

Tottman, D.R. & Wilson, B.J. (1990). Weed control in small grain cereals. In Hance, R.J. & Holly, K. (Editors): Weed Control Handbook. 8th Ed. Blackwell Scientific: Oxford: 301-328.

van der Vegte, W. (1978). Population differentiation and germination ecology in *Stellaria media*. Oecologia 37: 231-245.

van der Weide, R.Y. (1992). Phenology of arable and hedgerow populations of *Galium aparine* L. in relation to climate and soil conditions. Weed Research 32 (4): 249-258.

van der Weide, R.Y. (1993). Population dynamics and population control of *Galium aparine* L. PhD Thesis. Wageningen Agricultural University.

van der Weide, R.Y. & van Groenendael, J.M. (1990). How useful are population dynamical models? An example from *Galium aparine* L. Zeitschrift fur Pflanzenkrankheiton und Pflanzenschutz 12: 147-155.

van Gardingen, P.R., Curran, P.J. & Foody, G.M. (Editors) (1997). Scaling up: From cell to landscape. Cambridge University Press: Cambridge.

Vegis, A. (1964). Dormancy in higher plants. Annual Review of Plant Physiology 15: 185-224.

Vincent, E.M. & Roberts, H.A. (1977). The interaction of light, nitrate and alternating temperature in promoting germination of dormant seeds of common weed species. Seed Science and Technology 5: 659-670.

Vleeshouwers, L.M. (1997). Modelling weed emergence patterns. PhD Thesis: Wageningen Agricultural University.

Vleeshouwers, L.M. (1998). The effect of seed dormancy on percentage and rate of germination in *Polygonum persicaria* and its relevance for crop – weed interaction. Annals of Applied Biology 132: 289-299.

Vleeshouwers, L.M., Bouwmeester, H.J. & Karssen, C.M. (1995). Redefining seed dormancy: an attempt to integrate physiology and ecology. Journal of Ecology 83: 1031-1037.

Warren, J.M., Raybould, A.F., Ball, T., Gray, A.J. & Hayward, M.D. (1998). Genetic structure in the perennial grasses, *Lolium perenne* and *Agrostis curtisii*. Heredity 81: 556-562.

Warwick, S.I. & Marriage, P.B. (1982a). Geographical variation in populations of *Chenopodium album* resistant and susceptible to atrazine. I. Between- and within-population variation in growth and response to atrazine. Canadian Journal of Botany 60 (483): 493-493.

Warwick, S.I. & Marriage, P.B. (1982b). Geographical variation in populations of *Chenopodium album* resistant and susceptible to atrazine. II. Photoperiod and reciprocal transplant studies. Canadian Journal of Botany 60: 494-504.

Weaver, S.E., Tan, C.S. & Brain, P. (1988). Effect of temperature and soil moisture on time of emergence of tomatoes and four weed species. Canadian Journal of Plant Science 68: 877-886.

Wesson, G. & Wareing, P.F. (1969a). The role of light in the germination of naturally occurring populations of buried weed seeds. Journal of Experimental Botany 20 (63): 402-413.

Wesson, G. & Wareing, P.F. (1969b). The induction of light sensitivity in weed seeds by burial. Journal of Experimental Botany 20 (63): 414-425.

Westoby, M., Leishman, M.R. & Lord, J.M. (1995a). On misinterpreting the 'phylogenetic correction.' Journal of Ecology 83: 531-534.

Westoby, M., Leishman, M.R. & Lord, J.M. (1995b). Further remarks on phylogenetic correction. Journal of Ecology 83: 727-734.

Whytock, G.P. & Davies, D.H.K. (1996). The effect of time of application and herbicide dose on weed control in spring barley. Proceedings of Crop Protection in Northern Britain Conference: 171-176.

Williams, E.D. (1983). Effect of temperature, light, nitrate and pre-chilling on seed germination of grassland plants. Annals of Applied Biology 103: 161-172.

Williams, J.T. & Harper, J.L. (1965). Seed polymorphism and germination. I. The influence of nitrates and low temperatures on the germination of *Chenopodium album*. Weed Research 5: 141-150.

Wilson, B.J., Wright, K.J. & Butler, R.C. (1993). The effect of different frequencies of harrowing in the autumn or spring on winter wheat, and on the control of *Stellaria media* (L.) Vill., *Galium aparine* L. and *Brassica napus* L. Weed Research 33: 501-506.

Wiseman, A.J.L., Finch, H.J.S & Samuel, A.M. (1993). Crop husbandry including grassland. 7th Ed. Pergamon Press: Oxford.

Wulff, R.D. (1995). Environmental maternal effects on seed quality and germination. In Kigel, J. & Galili, G. (Editors): Seed development and germination. Marcel Dekker Inc.: New York: 491-506.

Ziska, L.H. & Bunce, J.A. (1993). The influence of elevated CO_2 and temperature on seed-germination and emergence from soil. Field Crop Reseach 34: 147-157.

Appendix 1: Standard potting compost

200 ℓ	Medium moss peat
100 ℓ	Perlite
120 g	Ammonium nitrate (34.5% NH_4NO_3) 'NitramExtra'
120 g	Potassium nitrate
675g	Calcium carbonate 'Garden lime'
675 g	'Dolodust' Enmag
120 g	'Frit'

Appendix 2: Seed dormancy and germination model definitions for Modelmaker (ver. 3.0.3.)

a) Model for seasonal changes in seed dormancy and identification of the germinable fraction, as described in section 11.5.1. and illustrated in Figure 11.6. Parameterised for the Leicestershire population and run at 5 °C and 0 MPa.

```
⊙t                          2000                0
▣Main
  ⊙aPsiT                    0.0754              0
    Controls the effect of  temperature x water potential on germination extent
  ⊙BetaTs                   50                  0
    Gradient of the sigmoid function sTs
  ▭fT  Unconditional
    Effect of temperature on germination extent
      fT = exp( -((Temperature-Topt)^2.0)/(2.0*sigmaT^2.0))
  ▭gWP  Unconditional
    Effect of water potential and water potential x temperature on germination extent
      gWP = exp(aPsiT*WaterPotential*Temperature)
  ▭Induction  Unconditional
    Rate of dormancy induction
      Induction = kI*sTs
  ⊙kI                       0.0925              0
    Intrinsic rate of dormancy induction
  ⊙kR                       0.0243              0
    Intrinsic rate of dormancy release
  ▭LowerCI  Unconditional
    Lower 95 % confidence interval for the proportion of seeds that are germinable at time t.  Based on
    Normal approximation to the binomial distribution.
      LowerCI = (pGerminable-1.96*sqrt(Variance))
  ⊙MuTs                     14.01               0
    Mean soil temperature for switch from dormancy release to dormancy induction
  ◇n  Unconditional
    Number of seeds
      n = 50
  ▭pGerminable  Unconditional
    Probability that a seed is germinable in given test conditions
      pGerminable = qtd*fT*gWP
  ⊙q0                       0.52                0
    Initial probability that a seed is not dormant
  ▭qtd  Unconditional
    Probability that a seed is not dormant at time t
      dqtd/dt = (1-qtd)*Release-(qtd*Induction)
      Initial Value = q0
  ▭Release  Unconditional
    Rate of dormancy release
      Release = kR*(1-sTs)
  ⊙SigmaT                   10.58               0
    Defines the width of the optimum temperature
  ▭sTs  Unconditional
    Effect of soil temperature on rates of dormancy induction and release
      sTs = exp(BetaTs*(Tmin-MuTs))/(1+exp(BetaTs*(Tmin-MuTs)))
  ◇Temperature  Unconditional
    Test temperature
      Temperature = 5
  ⊙Topt                     10.3                0
    Optimum temperature
```

```
Ts C:\Anna\Soiltemp2.txt
  Soil temperature at 10 cm
    t Control
    Tmin Controlled by: t Repeated
      Linear interpolation
    Tmax Controlled by: t Repeated
      Linear interpolation
UpperCI Unconditional
  Upper 95 % confidence interval for the proportion of seeds that are germianble at time t. Based on
  Normal approximation to the binomial distribution.
    UpperCI = (pGerminable+1.96*sqrt(Variance))
Variance Unconditional
  Variance in the proportion of seeds that are germinable at time t
    Variance = pGerminable*(1.0-pGerminable)/n
WaterPotential Unconditional
  Test water potential
    WaterPotential = 0
```

b) Model for timing of germination, as described in section 11.5.2. and illustrated in Figure 11.7. Parameterised for the Leicestershire population and run at 5 °C and 0 MPa.

```
t                    2000              0
Main
  aPsi               0.7112            0
    Controls the effect of water potential on hydrothermal time
  aPsiT              0.0754            0
    Controls the effect of  temperature x water potential on germination extent
  betaHT             0.0147            0
    Gradient of the sigmoid function hHT
  fT Unconditional
    Effect of temperature on germination extent and germination timing
      fT = exp( -((Temperature-Topt)^2.0)/(2.0*sigmaT^2.0))
  G Unconditional
    Expected number of seeds germinating at time t (assuming individual seeds germinate independently)
      G = pG*n
  g0                 0.0581            0
    Intrinsic germination rate
  gWP Unconditional
    Effect of water potential and water potential x temperature on germination extent
      gWP = exp(aPsiT*WaterPotential*Temperature)
  hHT Unconditional
    Effect of hydrothermal time on germination timing
      hHT = exp(betaHT*(HT-muHT))/(1.0+exp(betaHT*(HT-muHT)))
  HT Unconditional
    Hydrothermal time
      HT = t*Temperature*exp(aPsi*WaterPotential)
  LowerCI Unconditional
    Lower 95 % confidence interval for number of seeds germinating at time t. Based on Normal
    approximation to the binomial distribution.
      LowerCI = G-1.96*sqrt(VarG)
  muHT               894.27            0
    Mean hydrothermal time requirement
  n Unconditional
    Number of seeds
      n = 50
  pG Unconditional
    Probability that a seed germinates at time t
      pG = (1-pUG)*pGerminable
```

pGerminable Unconditional
Probability that a seed is germinable in given test conditions
pGerminable = q0*fT*gWP
pUG Unconditional
Probability of a germinable seed remaining ungerminated at time t
dpUG/dt = - g0*fT*hHT*pUG
Initial Value = 1
q0 0.724 0
Probability that a seed is not dormant
SigmaT 10.94 0
Defines the width of the optimum temperature
Temperature Unconditional
Test temperature
Temperature = 5
Topt 19.34 0
Optimum temperature
UpperCI Unconditional
Upper 95 % confidence interval for number of seeds germinating at time t. Based on Normal approximation to the binomial distribution.
UpperCI = G+1.96*sqrt(VarG)
VarG Unconditional
Variance in number of seeds germinating at time t
VarG = n*pG*(1.0-pG)
WaterPotential Unconditional